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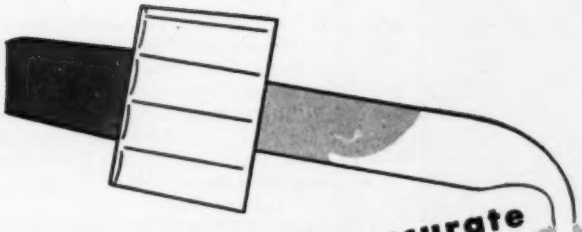

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ARCHIVES OF PATHOLOGY

VOLUME 56 JULY 1953 NUMBER 1

INVESTIGATION OF RADIUM DEPOSITION IN HUMAN SKELETON BY GROSS AND DETAILED AUTORADIOGRAPHY

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AND
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IT IS WELL established that certain radioactive elements are deposited principally in bone after they enter the body. The deposition is irregular and is accompanied by destructive changes. Information concerning the localization and effect of bone-seeking radioactive elements in the human body is sparse and has been derived chiefly from observations on persons who had been employed as luminous-dial painters. The damage that has been described cannot be assigned to any single radioactive element, since the luminous material contained varying amounts of radium, mesothorium, and radiothorium.

The materials for this investigation were obtained from two patients who were treated therapeutically with radium chloride and from one person who, between 1921 and 1927, worked as a luminous-dial painter.[1] This is the first time that large amounts of fresh human bone have been available for study of the precise pattern of distribution of radioactive elements that have been present in the body for as long as 24 years, and the material provides the first opportunity for an extensive study of the deposition and long-term effects of a single heavy radioactive element (radium).

The following are brief summaries of the case histories and include listings of the specimens taken for study:

CASE 1.—A 48-year-old white woman died in 1951 from a fibrosarcoma of the foot. She had been given radium chloride solution ("radium water") for a condition diagnosed as "migratory arthritis" 22 years prior to her death.

Specimens.—Femur, humerus, tibia, fibula, and skull.

CASE 2.—A 51-year-old white woman in 1951 had a reconstruction operation on the head of the femur for aseptic necrosis. Seventeen years before the operation she had been given intravenous injections of radium chloride for "mental depression."

Specimens.—Biopsy specimens from the greater trochanter, head of the femur, and fibula.

CASE 3.—A 48-year-old white woman who had worked as a luminous-dial painter from 1921 to 1927 died in 1951. She had multiple fractures of the femora, osteomyelitis of the mandible, and other complications which could be attributed to radium poisoning.

Specimens.—Samples from the vertebrae, ilium, and ribs.

From the Division of Biological and Medical Research, Argonne National Laboratory.

This investigation was undertaken while Dr. Looney was an Atomic Energy Commission Post Doctoral Research Fellow in the Medical Sciences of the National Research Council. His present address is Radioisotope Laboratory, U. S. Naval Hospital, National Naval Medical Center, Bethesda, Md.

1. It is probable that specimens from the luminous-dial worker contained mesothorium. As far as we have been able to ascertain only radium was present in the radium chloride solution.

AUTORADIOGRAPHIC TECHNIQUES

Gross Autoradiographs.—The technique described by Lotz and associates [2] was was simplified and used to prepare the gross autoradiographs.

All the bone specimens were placed in a deep-freeze unit after they were taken from the body. Just before sectioning they were removed from the freezer and kept in solid carbon dioxide. The bones were cut serially into 2 to 8 mm. slices with a band saw, and each section was cleaned with acetone to remove bone dust. Those sections that contained a relatively large amount of marrow were painted with a 1% solution of celloidin to minimize chemical fogging of the film. The bone slices were then placed between two sheets of no-screen x-ray film, and this layer was bound with rubber bands (care was taken to avoid pressure) and put on a board cut to size. Since several layers were to be stacked on each board, corrugated cardboard was inserted between successive layers to provide a level surface. Approximately five of these layers were placed in a porcelain tray, which was packed with creped wadding to prevent shifting. The tray's contents were sealed to exclude light by corrugated board taped over the top, and the tray was stored in the deep freeze. Gross autoradiographs were not used to determine the exact dimensions of the areas of concentration, because of the lack of resolution. Their main function was to provide (1) a comprehensive picture of the manner of distribution throughout the skeleton, (2) the approximate dimensions of individual areas, and (3) the localization of areas to be taken for detailed autoradiography.

Roentgenograms of the long bones and gross autoradiographs taken from these bones were used in selection of areas for detailed study. The site was located and mapped from the roentgenograms and autoradiograph, which were mounted simultaneously on an x-ray view box. These maps are excellent guides for sectioning and later serve as permanent records of the exact locations from which the specimens were taken.

Detailed Autoradiographs.—The autoradiographic method devised by Arnold [3] was found to be the most satisfactory for the material used in this investigation. The technique has two advantages: (1) serial sections, 6 to 12 μ thick, can be cut from blocks of undecalcified bone without distortion, and (2) the method of fixation does not leech radioactive elements from the specimens. Modifications, however, were necessary to provide cross sections of dense cortical bone. For these, a piece of bone was mounted on a platform, which could be moved in three planes, held in place with sharp tongs, and cut with a rotary saw.[4] This method gave sections 50 to 150 μ thick.

2. Lotz, W. E.; Gallimore, J. C., and Boyd, G. A.: How to Get Good Gross Autoradiographs of Large Undecalcified Bones, Nucleonics **10**:28-31 (March) 1952.

3. Arnold, J. S.: A Method for Embedding Undecalcified Bone for Histologic Sectioning, and Its Application to Radioautography, Science **114**:178-180 (Aug. 17) 1951. Progress Report: Radioautography, ANL-4625, Chicago, Argonne National Laboratory, 1951.

4. Roofe, P. G.; Hoecker, F. E., and Voorhees, C. D.: A Rapid Bone Sectioning Technic, Proc. Soc. Exper. Biol. & Med. **72**:619-622 (Dec.) 1949.

After the sections were cut they were covered with a photographic emulsion,[5] and the slides were placed in boxes that were sealed to light and humidity. Exposure time was varied in order to obtain the optimum time necessary to determine the manner of radium distribution without obscuring histological detail. After exposure the sections were overstained with hematoxylin, which served as a regressive stain. Then a 5% solution of aluminum ammonium sulfate was used to wash the stain from the emulsion.

OBSERVATIONS

Gross Autoradiographs

Skull.—Gross autoradiographs of the skull revealed small sharply defined areas of radium concentration distributed randomly throughout the coronal sections. These areas usually varied from a size so small that they could just be visualized to 2 mm. in diameter. There were also some diffuse areas (3 to 5 mm. in diameter), but these are believed to be the result of fusion of smaller ones. In general, radium appeared to be more concentrated in the diploe than in the denser surrounding bone (Fig. 1).

Humerus.—In the trabecular bone of the head of the humerus there were areas of concentration (1 to 3 mm. long and 1 to 2 mm. wide) adjacent to the articular surface, principally near the medial inferior border.[6] A 1 to 2 mm. area of less density was usually seen between the areas of concentration. Longitudinal areas of radium deposition (0.3 to 1 mm. wide and 1 to 3 mm. long) were scattered randomly throughout the cortex but occurred with greater frequency in the proximal half of the shaft. In addition, diffuse darkening of the film was observed in certain parts of the cortex.

Femur.—The gross autoradiographs revealed a linear concentration of radium 1 mm. thick outlining the entire head of the femur, and the greatest concentration was found around the inferior medial quadrant, just beneath the articular cartilage. Areas of concentration ranging in size from a pinpoint to 1 mm. in diameter were scattered throughout the bony structure of the femoral head, and these extended down into the neck and shaft as far as trabecular bone extended. There was usually a higher concentration of radium in the lower part of the femoral head and in the medial quadrant. In the cortex at the level of the lesser trochanter there was an area of concentration 1 by 4 cm. in size, and the superior surface of the greater trochanter was outlined by another area, 1 to 4 cm. in length and 3 mm. in width.

In the midsection of the medial aspect of the femoral shaft, near the periosteum, there was a 1 mm. by 10 cm. longitudinal area of concentration. Concentrations throughout the cortex (Fig. 2) were similar in appearance and distribution to those seen in the humerus. The concentrations occurred with greater frequency near the periosteum. In some sections, deposition near the endosteum and periosteum was heavy enough to outline the cortex. As in the humerus, the areas were greater in number in the proximal end of the shaft and in the cortex of the medial half of the shaft. A linear area of concentration, 1 mm. wide, surrounded the distal end of the femur, subarticularly and on its lateral sides. In the same area, there were small, denser concentrations having diameters of about 0.5 mm.

5. Gross, J.; Bogoroch, R.; Nadler, N. J., and Leblond, C. P.: The Theory and Methods of the Radioautographic Localization of Radioelements in Tissues, Am. J. Roentgenol. **65**:420-458 (March) 1951.

6. The humeral and femoral heads were arbitrarily divided into four quadrants to permit more accurate description of the location of radium-induced changes.

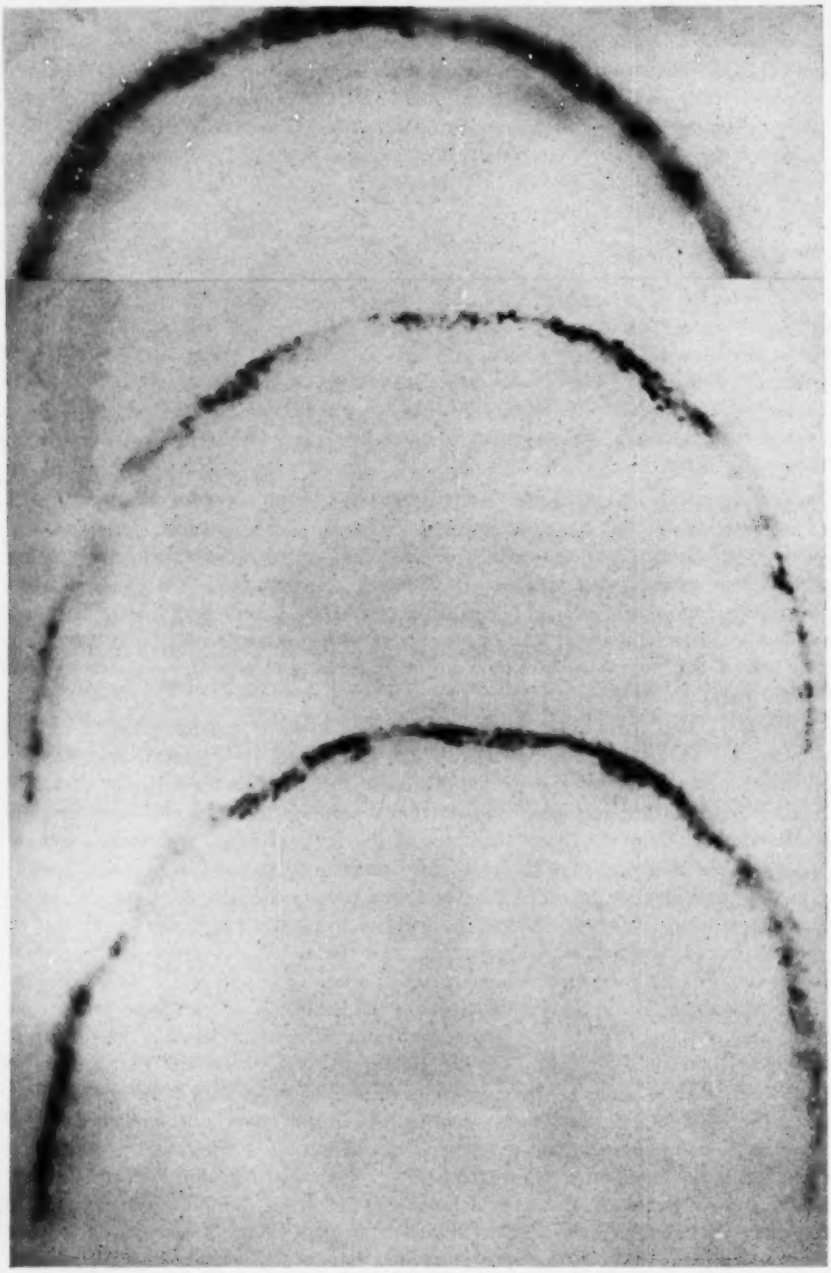

Fig. 1.—Gross autoradiographs of coronal sections of skull of Patient 1, exposed for five weeks. Note variation in size of areas of concentration and how, in certain places, they are grouped so closely together that they appear as one large concentration. Light area just to left of center of second section is probably due to poor contact on the film. In the center of the third section very small areas of concentration outline inner table.

Tibia.—The tibia also had a linear concentration of radium around the proximal end and subarticularly, and similar small, irregularly distributed areas were present in the trabecular bone. In several sections, these areas appeared with greater frequency in the metaphysis. A few gross autoradiographs revealed a vague horizontal outline of the sclerotic zone of the diaphyseal and epiphyseal union. The outline seemed to be narrower but more distinct at the distal end of the tibial bone.

In the cortex of the shaft of the tibia there were longitudinal areas of concentration that were similar to those already noted in the femur and humerus, but

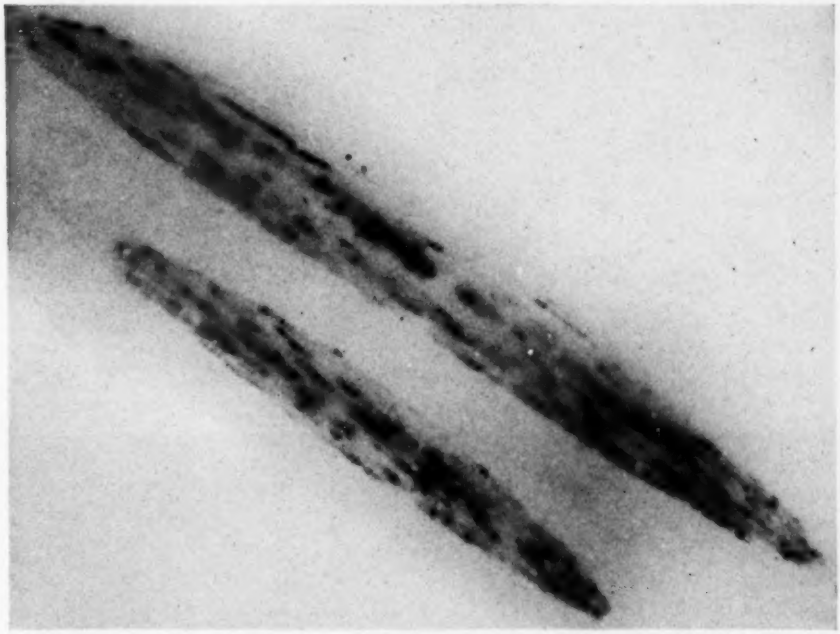

Fig. 2.—Autoradiographs of two longitudinal sections of cortex of femur from Patient 1, exposed for five weeks. Smaller section was taken 2 mm. from periosteum and larger section cut just medial to it. Areas of concentration are more frequent in smaller section and upper end of larger section, which were nearer periosteum. Large area of darkening in center of larger section is probably result of an endosteal concentration of radium, since this surface was on periphery of marrow cavity.

they were considerably less frequent. The only significant difference between deposition in the tibia and the other long bones was that almost all these concentrated areas were located near the periosteum (Fig. 3).

Fibula.—Almost all the areas of concentration appeared in the trabecular bone at the distal and proximal ends of the fibula, and only a few areas were noted in the cortex of the shaft. The five-week exposure period for specimens taken from the shaft of the fibula was not long enough to give a satisfactory picture of distribution.

Detailed Autoradiographs

Studies of the detailed autoradiographs of cortical bone revealed that radium was concentrated in about 10 to 15% of the Haversian systems (Figs. 4 and 5).

Within some Haversian systems, radium was confined primarily to one or two concentric lamellae (Fig. 4) around the periphery of the system or distributed throughout. Figure 4 shows a light concentration of radium in the interstitial lamellae between the two Haversian systems that have heavy concentrations, while the systems above and below are quite free of alpha tracks.

Fig. 3.—Autoradiograph of an entire longitudinal section of tibia from Patient 1, exposed five weeks, demonstrating irregularity of distribution of radium in trabecular bone. Small areas of concentration seen in proximal end extend down into shaft as far as trabecular bone extends. In distal end concentration is near articular surface. Vague outline of "sclerotic zone" of metaphyseal-epiphyseal junction cannot be seen in this section. Section does show linear area of concentration adjacent to articular cartilage. In cortex of shaft, areas of concentration are not frequent; however, they are more numerous in medial shaft and near periosteum of both shafts. Observe diffuse, less dense outline of shafts.

Darkening over the two concentric lamellae shown in Figure 5 clearly delineates the area of radium deposition in the system. A distinct linear concentration of radium deposition in an interstitial lamella is shown in Figure 6, with almost

Fig. 4.—Detailed autoradiograph of cross section of cortical bone from humeral shaft of Patient 1, exposed for five weeks. Two of small number of Haversian systems having radium concentrated in this section. Greatest concentration is in two concentric lamellae in center of Haversian system. Alpha tracks are less dense in remainder of Haversian systems and interstitial lamellae between systems. Observe that activity suddenly falls off around these areas of concentration and rest of photomicrographs are relatively free of alpha tracks. (× about 218.)

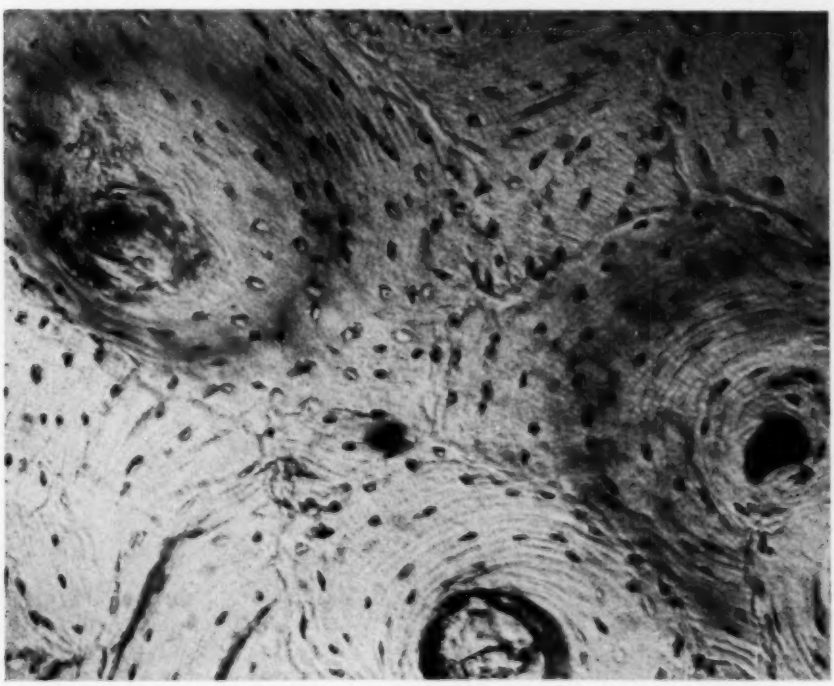

Fig. 5.—Photomicrograph of bone underlying detailed autoradiograph shown in Figure 4. Note dark concentric rings outlining lamellae having greatest concentration of radium. Central part of Haversian system in upper left is undergoing destructive changes. Figures 4 and 5 demonstrate how radium concentration and histopathological changes can be studied at same time. (× about 218.)

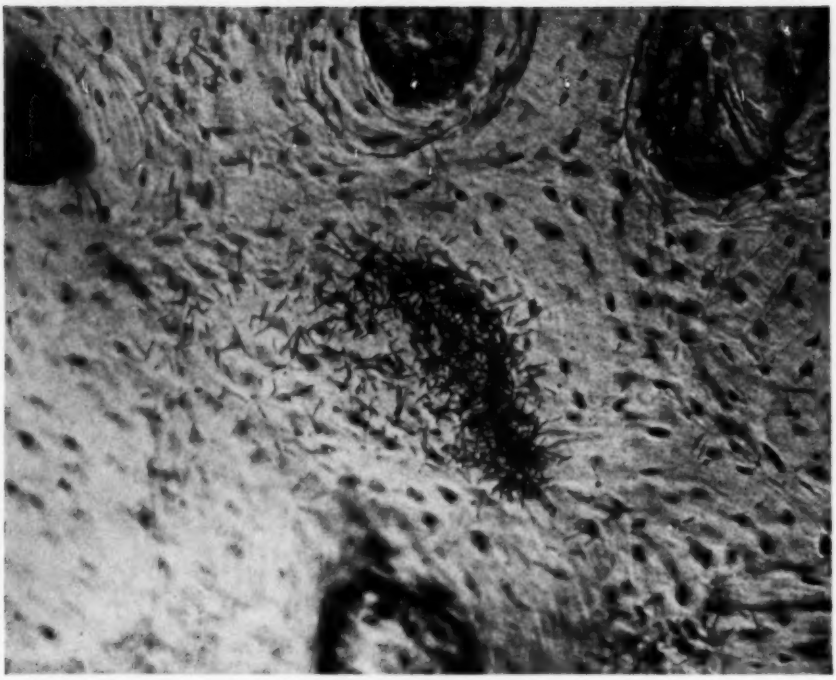

Fig. 6.—Area of concentration in interstitial lamella of the section represented in Figure 4. Observe that Haversian systems are almost entirely free of alpha tracks. Necrosis seen in upper right is proximal end of necrotic area 100 μ in length. ($\times$ about 218.)

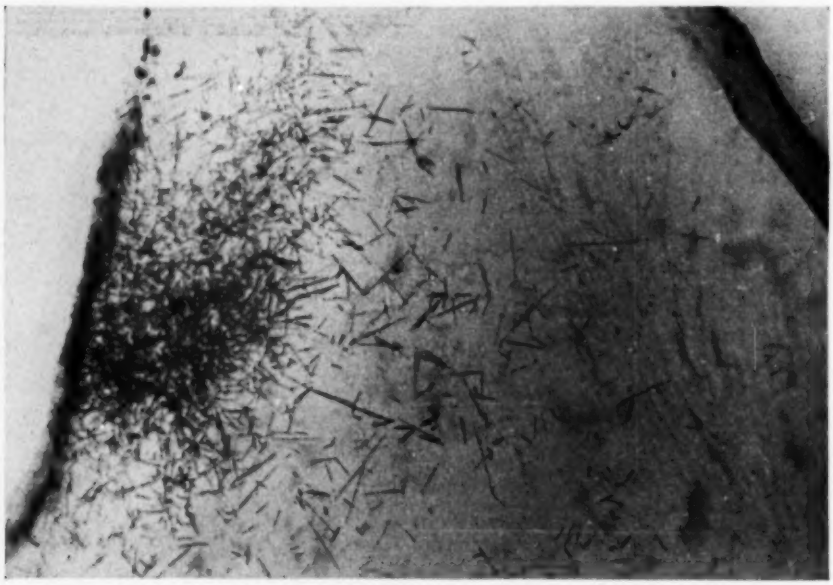

Fig. 7.—Base of large trabecula (Patient 2, 134-day exposure). Note area of heavy concentration of alpha radiation. Linear concentration beginning at top of this area extended for 50 to 60 μ parallel to curvature of trabecula. ($\times$ about 330.)

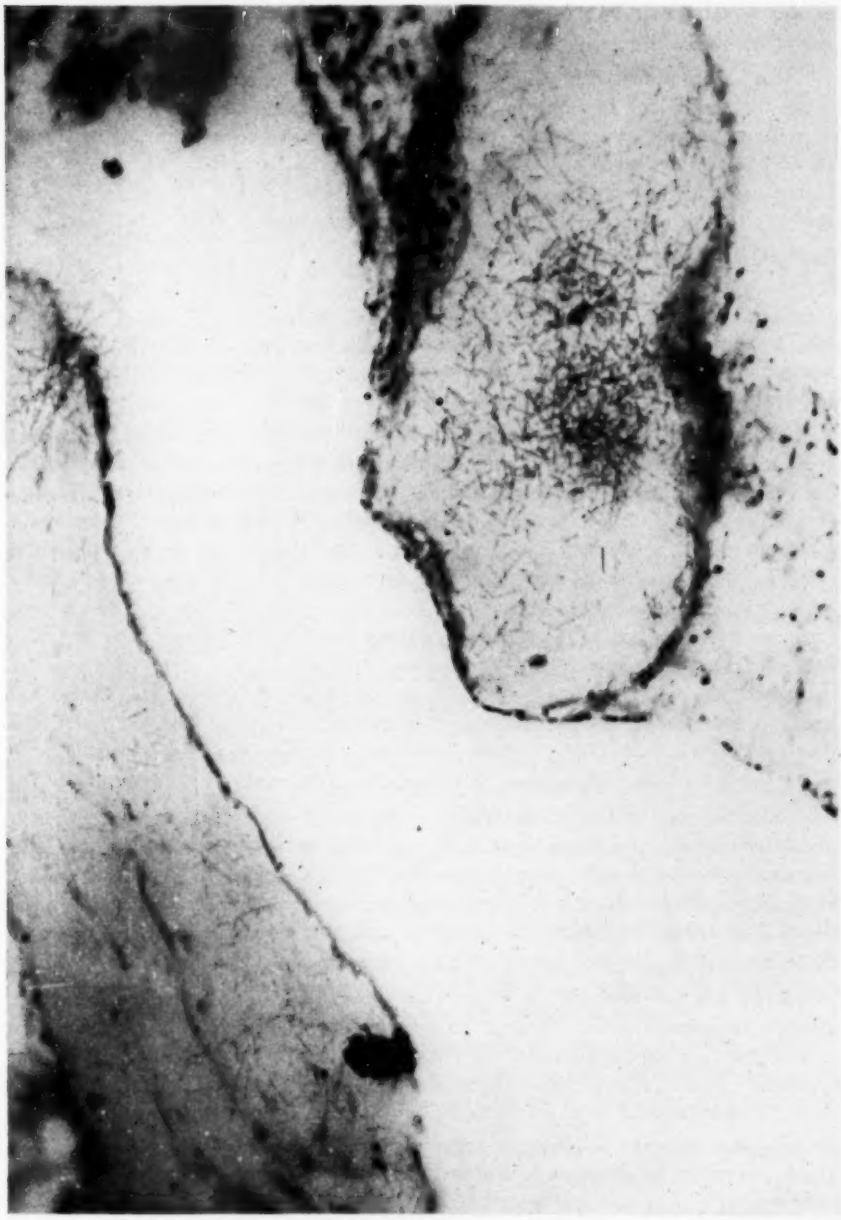

Fig. 8.—Patient 2, 134-day exposure. Trabecula in upper right has radium concentated in center. That on left has one small concentrated area at its superior border, with random distribution throughout. (× about 475.)

no evidence of deposition within the Haversian systems. The proximal end of an area of destruction, 100 μ long, is present in the upper right portion of this figure. No alpha tracks are present.

Areas of radium concentration in detailed autoradiographs of trabecular bone range from 5 to 15 μ in diameter (Figs. 7 and 8). There is a linear concentration of radium on the superior side of the area of greatest concentration that runs parallel to the curvature of the trabecula (Fig. 7). This is from 40 to 50 μ long. Linear areas of concentration were also noted in cementing lines. In some sections, several adjacent trabeculae had similar areas of concentration, while others appeared to be relatively free of radium.

COMMENT

The opportunity to study fresh human bone by means of gross and detailed autoradiographs provided a comprehensive picture of radium deposition in the human skeleton.

By this study of detailed autoradiographs made from sections causing darkened areas on the x-ray film, it was possible to confirm the fact that the darkened areas are the result of alpha particles emanating from radium. Darkened areas, usually measuring grossly 0.3 to 1 mm. in width and up to 15 mm. in length, were found to be the result of radium concentration in a small percentage of the Haversian systems and in interstitial lamellae. The dark linear area (1 mm. in thickness) outlining the contour of the ends of the long bones resulted from radium concentrations at the junction of the articular cartilage and bony structure. The irregular darkened areas throughout trabecular bone observed grossly were produced by concentrations of radium usually 5 to 15 μ in the greatest dimension. These areas had a wide variation in size, shape, and distribution, and they were found at any depth within the trabeculae. In some of the sections exposed for long periods there was a much less concentrated and more uniform distribution of radium.

The random distribution of radium in trabecular bone and the concentration of radium in the cementing lines and Haversian systems of compact bone are supporting evidence that radium is deposited in sites that are metabolically active at the time of administration or redistribution, while the more uniform and less dense distribution is probably the result of inorganic ion exchange.[7]

The uniformity of distribution within the Haversian system reported by Hoecker [8] has not been confirmed by this investigation. In some instances, the greatest concentration was confined to one or two concentric lamallae, with considerably less in the remainder of the Haversian system. This situation may be the result of (1) a longer period of deposition (Hoecker's observations are based on deposition periods of 7 to 10 years) and (2) differences in the selection of specimens.

In addition to the microscopic areas of destruction shown in the photomicrographs, macroscopic areas of destruction have been observed in compact bone 1 to

7. Schubert, J.: Estimating Radioelements in Exposed Individuals, Nucleonics **8**:13-28 (Feb.) 1951.

8. Hoecker, F. E., and Roofe, P. G.: Studies of Radium in Human Bone, Radiology **56**:89-98 (Jan.) 1951.

2 mm. in width and 5 to 20 mm. in length.[9] As previously shown, microscopic destructive changes within the Haversian system were found in the presence and absence of radium concentrations. In almost every instance, however, radium was rarely present in or around the macroscopic areas of destruction. Thus far, areas of transition, in which both macroscopic change and radium concentration appear together, have been found very infrequently. It is not possible, therefore, to draw any conclusion regarding a direct relation between these macroscopic areas of destruction and the radium concentrations, even though the dimensions are of the same order of magnitude. It has been postulated that these macroscopic areas of destruction occur as a result of the fusion of adjacent central canals of Haversian systems undergoing degenerative changes.[10] It has been further postulated that the radium has been removed from the macroscopic areas of destruction by the time the changes occur.[11] It is evident that considerable difficulties are inherent in any attempt to reconstruct a pathological process [12] which has been going on for 20 to 30 years from specimens taken at the termination of this process.

At present considerable emphasis has been placed on the difference in the late effect seen in luminous-dial workers and persons given radium chloride ("radium water"). No difference was noted in either autoradiographic findings or pathological changes in the specimens from the luminous-dial worker and the patients who received radium chloride.

SUMMARY AND CONCLUSIONS

A comprehensive and precise pattern of the mode of deposition of radium in the human skeleton has been obtained. Radium was found chiefly in small areas of heavy concentration irregularly distributed in both compact and trabecular bone.

In compact bone only about 10 to 15% of the Haversian systems and interstitial lamellae had concentrations of radium. In a larger number of instances most of the radium was confined to one or two concentric lamallae.

The areas of radium concentration in trabecular bone were usually 5 to 15 μ in the greatest dimension. However, there was a wide range in size and shape of these areas, and they were found at any depth within the trabeculae. In some instances linear concentrations ran parallel to the curvature of the trabecula for 50 to 100 μ. Heavy and fairly uniform concentrations of radium were present at the junction of the articular cartilage and the trabeculae of the long bones and vertebrae. They appeared as a 1 mm. linear outline of the contour of the bone in the gross autoradiographs. Some cementing lines were clearly outlined by heavy concentrations of radium. In some sections exposed for long periods there was a much less concentrated and more uniform distribution of radium.

These findings are in agreement with existing theories that radium has more than one principal mode of deposition. The small, highly concentrated areas may have been areas in which bone formation was taking place at the time of admin-

9. Looney, W. B.: A Progress Report: Clinical Studies on 24 Patients, ANL-4666, Chicago, Argonne National Laboratory, 1951.

10. Phemister, D. B.: Personal communication to the author.

11. Aub, J. C.; Evans, R. D.; Hemplemann, L. H., and Martland, H. S.: Late Effects of Internally Deposited Radioactive Materials in Man, Medicine **31**:221-329 (Sept.) 1952.

12. A more detailed report of the histopathological changes is in the process of being completed.

istration or redistribution. The more uniform and less dense distribution may be the result of inorganic ion exchange.

Microscopic areas of destruction were found in the presence or absence of concentrations of radium. In almost every instance radium was not present in or around macroscopic areas of destruction. It is evident that some relation exists between radium deposition and bone destruction. However, no conclusion can be drawn about a direct relation at present.

Certain postulates are presented which attempt to explain the manner in which radium has produced destructive changes in the skeleton.

EFFECT OF HEMOLYTIC TOXIN OF STREPTOCOCCUS PYOGENES ON VIRAL MYOCARDITIS IN RABBIT

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IF A VIRUS is inoculated at a peripheral site such as the skin, the testis, or the upper respiratory tract into a rabbit that has been suitably prepared by specific preceding or concurrent procedures, the infective agent will reach the heart, lodge in that organ, and there produce a characteristic lesion.[1] This ability to produce cardiac lesions is not an attribute of a single virus but is common to many to which the rabbit is susceptible. In the initial experiments virus III was used exclusively,[1] but later the myxoma virus, two strains of fibroma virus, and the viruses of vaccinia and pseudorabies were found to behave similarly in respect to cardiac localization, although each produced its own typical histologic response.[2] There is also a functional alteration that has been demonstrated by changes in the electrocardiograms of the infected animals.[3]

The predominant lesion is myocarditis, but not uncommonly this is accompanied by valvulitis, endocarditis, or pericarditis. These lesions resulting from infection with virus III or pseudorabies virus can be identified by the presence in them of cells containing the intranuclear inclusion bodies characteristic of these diseases (Figs. 1 and 2). The distinctive cellular response to the myxoma and fibroma infections also indicates their viral origin. This morphological evidence of the viral etiology of the cardiac lesion is substantiated by the fact that it is possible to transmit the disease to another animal by the injection of a saline suspension of the ground-up heart.

The frequency of occurrence of myocarditis and its severity are dependent upon the submission of the inoculated animal to some preparatory or predisposing procedure that increases the susceptibility of the heart to viral infection. The intravenous injection of a variety of substances—gum acacia, vasopressin injection (Pitressin), epinephrine, barium chloride—differing widely in pharmacologic and

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From the Departments of Surgery and Pathology, Cornell University Medical College, and the New York Hospital.

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2. Pearce, J. M.: Susceptibility of the Heart of the Rabbit to Specific Infection in Viral Disease, Arch. Path. **34**:319-333, 1942.

3. Pearce, J. M., and Levine, H. D.: The Anatomic Causes of Electrocardiographic Changes in Virus Myocarditis of Rabbits, Am. Heart J. **25**:102-111, 1943.

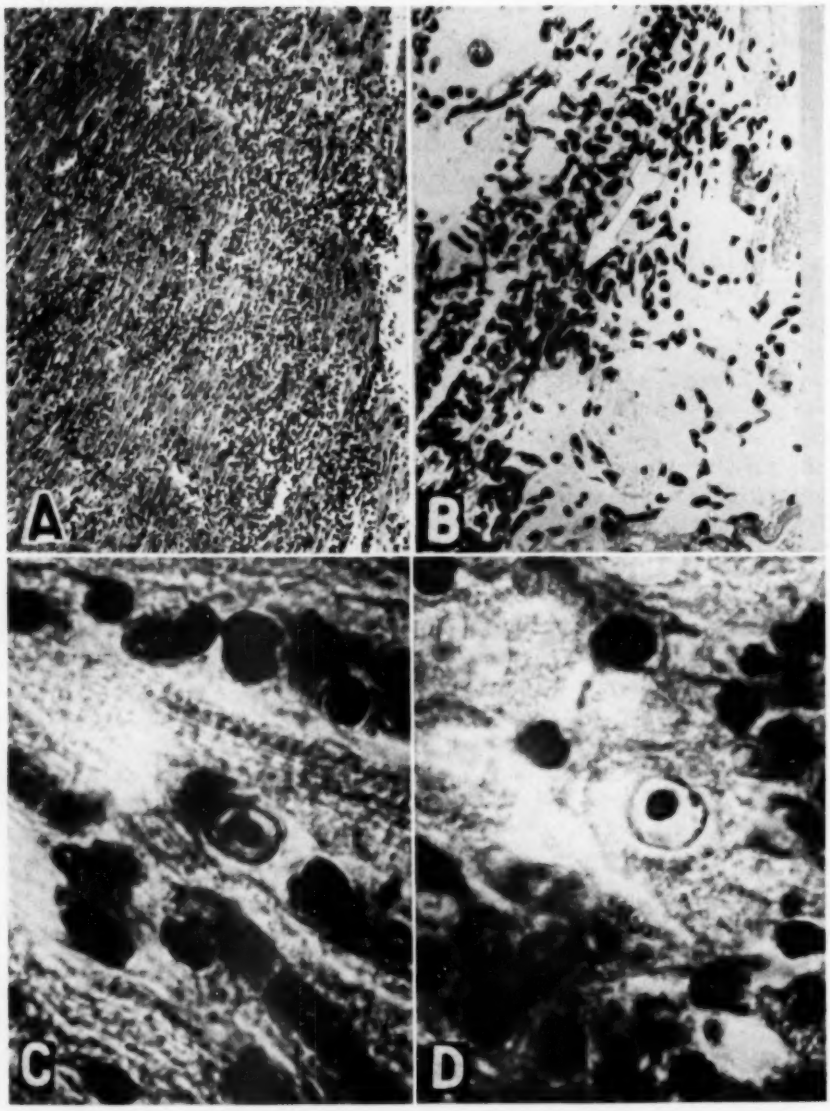

Fig. 1.—Virus III myocarditis in rabbits prepared by intravenous vasopressin injection (Pitressin) or gum acacia. *A*, diffuse leucocytic infiltration of myocardium; × 70. *B*, collection of leucocytes in myocardial interstitial spaces. Note intranuclear inclusion body in center of field; × 400. *C*, intranuclear inclusion body in mononuclear cell between muscle fibers; × 1,450. *D*, intranuclear inclusion body in nucleus of cardiac muscle cell; × 1,450.

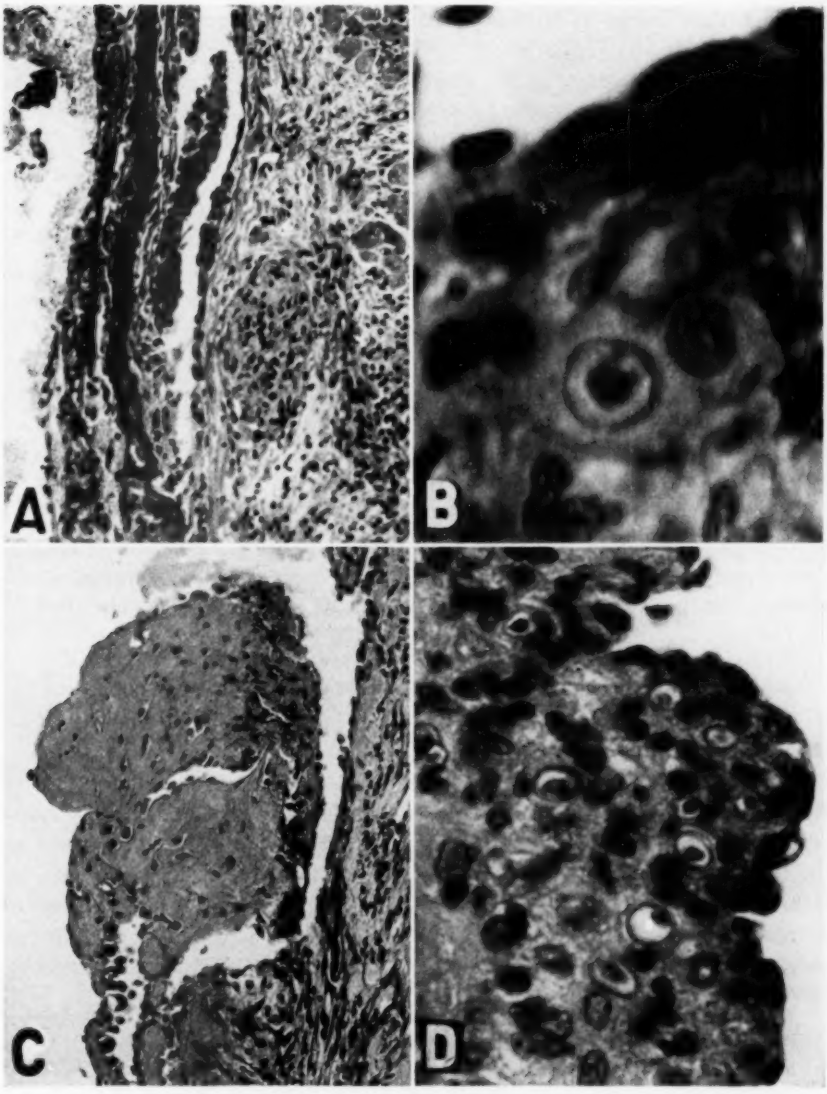

Fig. 2.—Virus III pericarditis and endocarditis. *A*, an exudate of fibrin and a few leucocytes overlying the epicardium of the inflamed left ventricle; × 150. *B*, intranuclear inclusion body in epicardium; × 1,450. *C*, fibrinoid vegetation at base of mitral valve; × 150. *D*, intranuclear inclusion bodies in substance of mitral valve; × 800.

physical properties has been used successfully for this purpose. An investigation of the mechanism by which these chemically unrelated materials induced the cardiac localization of virus led to the hypothesis that it lay in their one common attribute, their ability to decrease the quantity of oxygen supplied to the heart, and that a relative cardiac anoxia, or hypoxia, was the factor that enabled a circulating virus to lodge in the heart and there produce severe lesions. The theory that hypoxia was at least one determining factor was then proved conclusively in experiments duplicating the previous results by directly submitting virus-inoculated animals to an atmosphere deficient in oxygen. In these, too, extensive myocarditis developed.[4]

Although the products of Streptococcus pyogenes have not been shown to produce cardiac ischemia, the long-standing clinical observation that attacks of acute rheumatic heart disease in humans are frequently preceded by streptococcal pharyngitis or tonsillitis and the repeated observation [5] that a rise in the titer in the blood of the antibodies to the streptococcus and its toxins is concomitant with recurrence of acute rheumatic fever suggested that the products of that organism also might play a part in the production of an experimental viral heart lesion. This suspicion was strengthened by the recent experiments of Murphy and Swift in which they demonstrated inflammatory changes in the hearts of rabbits repeatedly infected with hemolytic streptococci.[6]

The experiments to be described in this paper were therefore instituted to determine the effect of a hemolytic toxin of Str. pyogenes on the incidence and severity of experimental viral carditis in the rabbit.

METHOD AND MATERIAL

As in the majority of the preceding studies, virus III was the infecting agent. The reasons for this choice are several. Most important is the fact that lesions caused by this virus can be identified by the presence in them of cells containing the easily recognizable, large acidophilic intranuclear inclusion bodies typical of those classified by Cowdry as Type A.[7] The discovery of these bodies in tissue sections of the hearts of infected animals is sufficient evidence that the virus is in the lesion and does away with the necessity for the more tedious procedure of animal inoculation to prove its presence. In addition, this virus is pathogenic to no species other than the rabbit, so that the danger of accidental laboratory and animal house infection is obviated, and also it is so low in virulence that, although

4. Pearce, J. M., and Lange, G.: Cardiac Anoxia as the Factor Determining the Occurrence of Experimental Viral Carditis, Arch. Path. **44**:103-112, 1947.

5. Coburn, A. F., and Pauli, R. H.: Studies on the Immune Response of the Rheumatic Subject and Its Relationship to Activity of the Rheumatic Process, J. Exper. Med. **62**:129-178, 1935.

6. Murphy, G. E., and Swift, H. F.: Induction of Cardiac Lesions, Closely Resembling Those of Rheumatic Fever, in Rabbits Following Repeated Skin Infections with Group A Streptococci, J. Exper. Med. **89**:687-698, 1949.

7. Cowdry, E. V.: A Comparison of the Intranuclear Inclusions Produced by the Herpetic Virus and by Virus III in Rabbits, Arch. Path. **10**:23-37, 1930.

practically all rabbits in the available breeding stocks are susceptible to it,[8] premature deaths of the inoculated experimental animals from infection per se do not occur.

The virus injection was made into the testis in amounts from 0.5 to .2 cc. The material used was a 5 or 10% suspension made by grinding the aseptically removed testis of a rabbit (similarly inoculated 4 or 5 days before) in a mortar with sterile sand and 0.85% sodium chloride solution.

All the rabbits were young males weighing between 1,700 and 4,000 gm., the majority weighing about 2,500 gm. They were unselected as to breed or color and had been acquired from a variety of sources.

The Str. pyogenes toxin was provided by Dr. Alan W. Bernheimer of New York University, College of Medicine, and prepared by him according to the methods he described in 1942[9] and 1945.[10] He has demonstrated that this toxin is similar to, if not identical with, streptolysin O. Immediately before use it was activated at a cold temperature with 1% cysteine. The hemolytic potency of the toxin concentrate was about 100,000 units per cc. (the unit being the amount of hemolysin that will liberate half the hemoglobin in 1 cc. of a 0.7% suspension of washed human erythrocytes) in a final volume of 2 cc. Injections of 0.025 cc. to 0.05 cc per kilogram were made into the marginal ear vein.

Twenty rabbits received both virus and toxin, the intravenous injection being made immediately after the intratesticular injection. Twenty-two rabbits received toxin alone. One hundred fifty-eight animals studied in previous experiments served as a control group that had been inoculated with virus but had undergone no other procedure.

All the animals either died or were killed in four to eight days. Complete autopsies were done immediately after death and always included histologic examination of heart, kidney, and testis. The tissues were fixed in Zenker's fluid and stained routinely with hematoxylin and eosin. The hearts were examined externally but were not opened since the microscopic appearance is more significant than the gross, and it had been found in earlier work[1] that more satisfactory sections for histologic study could be obtained by fixing the intact organ, then trimming it, and embedding it in paraffin in such a way that valves and chambers appeared in their natural relationship.

RESULTS

The results of these experiments are summarized in the Table.

Myocarditis in Rabbits Given Injections of Streptococcus Toxin and Virus III

	Toxin Alone	Virus Alone	Toxin and Virus
No. of rabbits	22	158	20
Rabbits with lesions	9 (41%)	16 (10%)	14 (66%)
Rabbits with severe lesions	0	0	6 (30%)
Lesions with inclusion bodies	0	158 (100%)	20 (100%)

In nine of the 22 animals whose sole treatment was the administration of the streptococcus toxin demonstrable lesions developed, but these lesions were never severe or extensive. They consisted of sparse focal infiltrations of lymphocytes

8. Pearce, J. M.: A Failure to Find Virus III Infection in Rabbit Breeding Stocks, Proc. Soc. Exper. Biol. & Med. **38**:872-873, 1938.

9. Bernheimer, A. W., and Pappenheimer, A. M., Jr.: Factors Necessary for Massive Growth of Group A Hemolytic Streptococcus, J. Bact. **43**:481-494, 1942. Bernheimer, A. W.; Gillman, W.; Hottle, G. A., and Pappenheimer, A. M., Jr.: An Important Medium for the Cultivation of Hemolytic Streptococcus, ibid. **43**:495-498, 1942.

10. Bernheimer, A. W., and Cantoni, G. L.: The Cardiotoxic Action of Preparations Containing the Oxygen-Labile Hemolysin of Streptococcus Pyogenes: Increased Sensitivity of Isolated Frog's Heart to Repeated Application of Toxin, J. Exper. Med. **81**:295-306, 1945.

and other mononuclear cells or of small subendocardial or intramyocardial extravasation of erythrocytes (Fig. 3). Rarely individual muscle fibers in these areas seemed swollen and somewhat more acidophilic than their neighbors, but it was not possible to make a definite diagnosis of necrosis. Obviously none of these lesions contained inclusions and all were entirely nonspecific in appearance.

The hearts of the remaining 13 animals (59%) in this group were entirely devoid of anatomic alteration.

Of the large control group that has been collected over a period of years and that consisted of animals inoculated with virus III but not otherwise treated,

Fig. 3.—A small collection of lymphocytes and extravasated erythrocytes that is typical of the lesion in rabbits that had received a single intravenous injection of the streptococcus toxin only. The inflammatory foci are rarely larger and always sparse; × 450.

cardiac lesion developed in 16 (10%) of the 158 animals in which the presence of intranuclear inclusion bodies indicated viral etiology. The myocarditis in these hearts was always minimal, and often the areas of inflammation were difficult to find.

A much larger proportion, 14 (66%), of the hearts of the 20 animals that received both toxin and virus contained inflammatory foci, and, even more significant, in half of these the lesions were extensive and striking. In all of these animals there were the intranuclear inclusion bodies that characterize virus III infection. The areas of mononuclear leucocytic infiltration were denser and more extensive (Fig. 4). Often there was obvious necrosis of muscle fibers and much

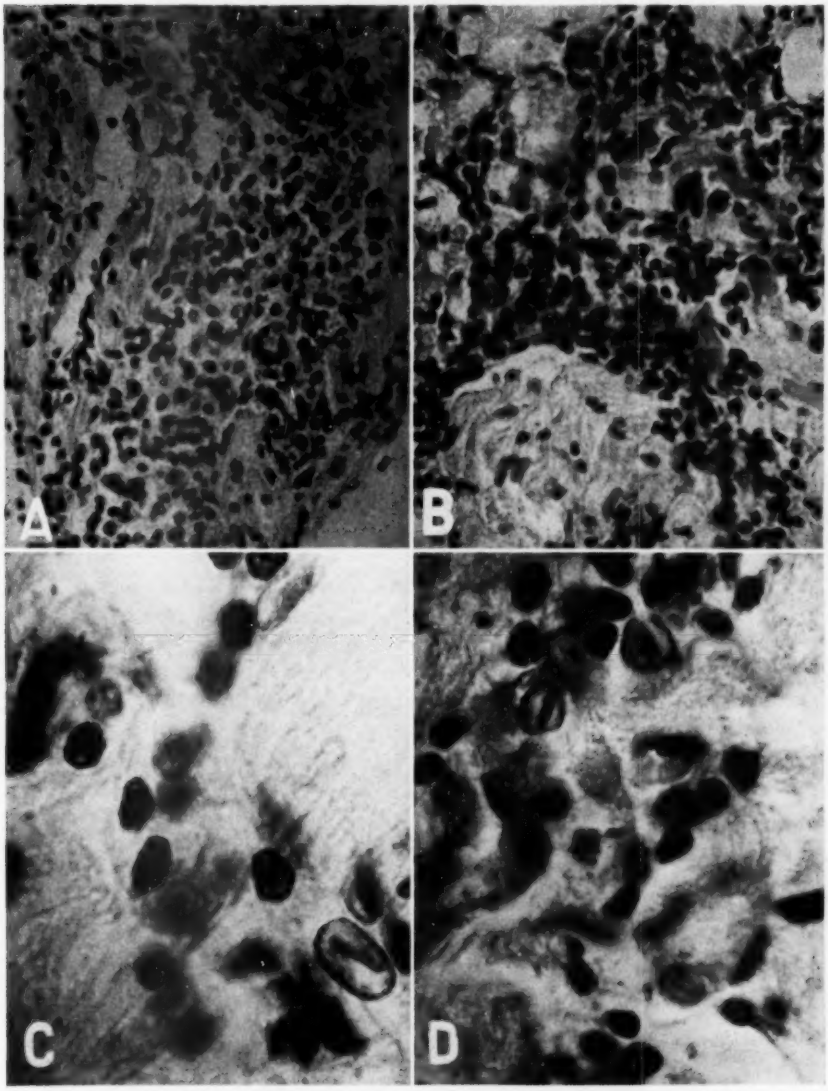

Fig. 4.—Virus III myocarditis in rabbits that had also had a single intravenous injection of hemolytic streptococcus toxin. *A* and *B*, leucocytic infiltration of the myocardium; × 300 (*A*) and × 370 (*B*). *C* and *D*, intranuclear inclusion bodies in the inflamed areas; × 1,000.

Fig. 5.—Area of necrosis, calcification, and inflammation in the ventricular walls of the heart of a rabbit infected with virus III and given injection intravenously with the streptococcus toxin; × 75.

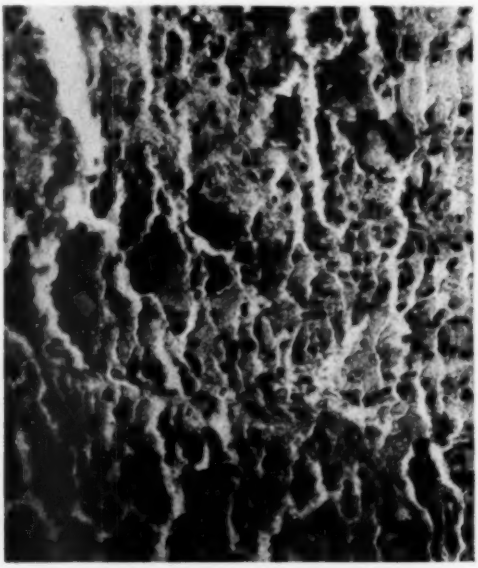

Fig. 6.—Calcification and necrosis in the left ventricle. These lesions are also accompanied by intranuclear inclusion bodies. This is the same heart illustrated in Figure 5; × 350.

calcification (Figs. 5 and 6). Although there was no instance of vegetative endocarditis or even of interstitial valvulitis there were many areas of localized endocardial thickening, and it is in these especially that inclusions were encountered.

COMMENT

From these experiments it appears that a hemolytic toxin of Str. pyogenes, when injected into the blood stream of a rabbit that has been inoculated just previously with virus III, induces that virus, which must also have entered the blood stream, to localize in the heart and there produce a myocarditis. The mechanism of this action is still to be elucidated. If the theory based on the earlier experiments,[4] that cardiac hypoxia is the chief factor in bringing about cardiac localization of virus, is accepted, three possible explanations come to mind. The simplest of these is that this hemolytic toxin may have a pharmacologic action on the coronary circulation similar to that of barium chloride and may thereby cause a spastic contraction of these arteries that in turn deprives the myocardium of oxygen-carrying blood. In addition, it may, like epinephrine, so increase the contractile force of the heart and thus the intramyocardial pressure that blood is prevented from flowing through the smaller arterioles and capillaries in the muscle mass. Indirect evidence in favor of this view is the demonstration by Bernheimer and Cantoni [10] that similar preparations of this oxygen-labile hemolysin of Str. pyogenes bring the isolated frog heart to a standstill in systolic contracture.

A slightly more complicated hypothesis is that the toxin interferes with the normal activity of the respiratory enzymes in the muscle cell and thereby produces a cellular hypoxia that allows virus infection.

The third possibility is that the toxin causes death of isolated bits of muscle in a less specific manner and that these necrotic muscles constitute localized areas of "cardiac hypoxia" that are susceptible to viral infection. This last and least specific explanation accords well with the fact that visible lesions did occur in the rabbits given only toxin. These may well have been transient and were certainly minimal alterations. Only when the self-perpetuating and possibly more powerful noxious agent the filtrable virus was superimposed, did they reach notable severity.

Although the toxin is a strongly hemolytic substance it seems unlikely that in the amounts used it could have caused sufficient intravascular hemolysis to bring about myocardial hypoxia, because there were no hemoglobin casts in the renal tubules, no hemoglobin in the excreted or bladder urine, and no staining of aortic intima.

SUMMARY

The intravenous administration of a single small dose of an oxygen-labile hemolysin of Streptococcus pyogenes (streptolysin O) occasionally produces in the heart of the rabbit a mild focal myocarditis characterized by sparse lymphocytic infiltrations and small extravasations of erythrocytes.

In rabbits infected with virus III by inoculation into the testis or some other peripheral site infrequently a mild myocarditis develops, caused by the virus entering the circulation and lodging in the heart.

When the inoculation of the animal with virus III is followed by the injection of the streptococcus toxin, not only is the incidence of the myocarditis greatly increased and its severity greatly augmented but also the inflammatory reaction is again characterized by the presence of cells containing the large intranuclear inclusion bodies typical of virus III infection.

The streptococcus toxin has the same effect, although to a lower degree, in causing cardiac localization of virus and in increasing the magnitude of the viral lesion in the heart as do the other substances that have been thought to act through the production of myocardial anoxia or hypoxia. Possible explanations for this action are discussed.

ACUTE DIFFUSE VASCULAR DISEASE ELICITED BY RENIN IN RATS PRETREATED WITH CORTISONE

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INJECTION of renal extracts containing renin (hereafter referred to as renin) precipitates a syndrome of edema, oliguria, and convulsions in rats pretreated with desoxycorticosterone acetate and sodium chloride.[1] The syndrome has some of the appearance of eclamptogenic toxemia of pregnancy. At autopsy the animals show edema, anasarca, and visceral hemorrhages; microscopically they present an acute vascular disease with capillary "colloid-looking" thrombi in the kidney and brain, as well as foci of necrosis in the myocardium and intestinal wall; degenerative changes are especially severe in the glomeruli.[2] When instead of giving desoxycorticosterone and isotonic salt, the animals are maintained for long periods on hypertonic saline, injection of renin precipitates a similar milder clinical state and minimal vascular lesions.[1]

The clinical implications of this "desoxycorticosterone-renin disease" are diminished by the consideration that desoxycorticosterone is not accepted as an equivalent of natural adrenal cortical hormone and that ingestion of hypertonic sodium chloride is highly abnormal. However, as will be shown, cortisone, a natural adrenal hormone, can substitute for desoxycorticosterone in sensitizing rats to renin. Thus the possibility persists that these experimental diseases may have equivalents in human disorders.

METHODS

Infection and cachexia cause a high mortality in rats of 100 gm. body weight or less when they are given cortisone over long periods. Consequently, animals of 150 to 200 gm. body weight were selected for this study. These were uninephrectomized and placed on a diet of Purina Fox Chow with 1% sodium chloride solution as drinking fluid. Out of 56 animals used, half were given cortisone acetate (2.5 mg. daily, subcutaneously), while the other half remained untreated.

At the end of 15 days, all animals were placed in metabolism cages and divided into four groups of 14 animals each, treated as follows: Group I, cortisone plus renin; Group II, cortisone;

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From the Research Division of the Cleveland Clinic Foundation, and the Frank E. Bunts Educational Institute.

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2. Masson, G. M. C.; Corcoran, A. C., and Page, I. H.: Renal and Vascular Lesions Elicited by "Renin" in Rats with Desoxycorticosterone Hypertension, A. M. A. Arch. Path. **53**:217, 1952.

Group III, renin, and Group IV, no treatment. Renin [3] was given in three daily subcutaneous injections of 0.5 cc. each (approximately 30 Goldblatt dog units per dose) for three days. At the end of this time, or at death, the animals were autopsied. Organs selected for examination were fixed in Zenker's fluid, weighed, sectioned, and stained with hematoxylin-eosin-methylene blue, periodic acid-fuchsin, and Masson's trichrome stains.

During the treatment period the functions observed were body weight, urine volume, blood pressure (determined by recording from tail pulse by an adaptation of the method of Olmsted and others [4]), and rectal temperature, as measured with a themocouple.

RESULTS

Physiologic and Metabolic Changes.—While the data are most conveniently assembled as means, it must be noted that means in the experimental groups, particularly in the cortisone-renin group, are not necessarily representative of changes of widely variable severity.

During the first 15 days of the experiment, that is, prior to administration of renin, the cortisone-treated rats lost an average of 13 gm. in body weight, while the nontreated animals grew normally and gained an average of 25 gm. Blood pressure

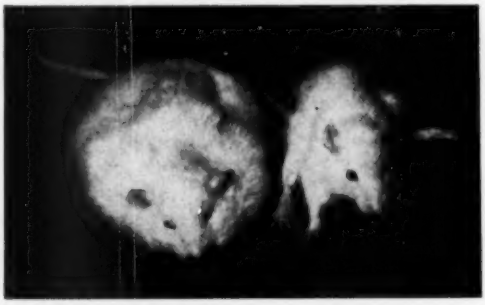

Fig. 1.—Cortisone-treated (right) and cortisone-renin-treated (left) rats of equal body weight at the time the latter was given renin.

in the cortisone group averaged 173 mm. Hg (range 150 to 198), as compared with a mean of 138 mm. Hg (118 to 158) in the control group.

Administration of renin had no remarkable effect in groups other than Group I (cortisone plus renin), in which two rats died within the first 24 hours; on the last day of the experiment, another rat was dead and two were comatose. In the same group, more than two-thirds of the animals gained weight as a result of fluid retention, which could be detected as early as eight hours after beginning of renin treatment; one rat gained 60 gm. within the first 24 hours (Fig. 1). Fluid retention was accompanied by decreased body temperature: It averaged 93.6 F. (86.4 to 96.8 F.) in Group I and 97.5 F. (96.8 to 98 F.) in the untreated controls (Group IV). Cortisone alone did not alter urine flow, which averaged 19 cc. daily in Group II and 16 cc. in Group IV. On the other hand, renin alone (Group III) caused a marked diuresis (mean urine flow 95 cc., range 40 to 180 cc. daily) in most of the

3. The renin used was the same as that described in previous experiments [2] and was prepared by Dr. A. A. Green.

4. Olmsted, F.; Corcoran, A. C., and Page, I. H.: Blood Pressure in Unanesthetized Rat, Circulation **3**:722, 1951.

animals, as compared with those which received cortisone plus renin (Group I); in the latter group, urine flow averaged 61 cc. (range 20 to 117 cc. daily) and hematuria was noted in two of the rats. The three animals of Group I in which urine flow increased did not show fluid retention. Levels of blood pressure as high as 240 mm. Hg were recorded in some of the rats of the cortisone plus renin group; with the onset of severe edema, the apparent pressure in the tail decreased to about 125 mm. Hg; this seeming decrease in pressure may be an artefact due to edema of the tail. None of the animals showed nervous symptoms.

Morphologic Changes.—Gross: The organs of rats given renin alone were grossly normal. Pulmonary abscesses were present in some of the animals treated with cortisone or with cortisone followed by renin. These were much less severe and common in the rats used in these experiments than in the smaller animals studied during preliminary experiments.

Specific gross lesions were present only in the rats of the cortisone-renin series. These manifested fluid retention (accumulations of peritoneal, pleural, pericardial fluid; edema of pancreas, mesentery, and intestinal tract; pulmonary edema, and congestion of variable degree) and hemorrhages. The latter were noted in heart, stomach, and intestines. In the heart they were massive and diffuse through the auricles and in the right ventricle. In the intestine they occurred as petechiae or as larger areas, some of which ulcerated, filling the bowel with blood; those of the mesoappendix were severer, commonly ulcerating, and extending through the muscularis so that they were visible through the serosa. No sites of bleeding were demonstrable in the kidneys, although some of the animals had shown hematuria, and no hemorrhages were found in the brain.

The kidney weights were similar in all groups, both absolutely and as percent of body weight prior to renin administration. However, the hearts of the cortisone-renin animals were heavier than those of untreated controls; the mean weight was 0.32 gm. (range 0.27 to 0.38 gm.) in experimental animals as compared with 0.26 gm. (range 0.24 to 0.29 gm.) per 100 gm. body weight in controls. The P value of this difference is less than 0.05. As expected, treatment with cortisone caused adrenal atrophy, with mean weights in Groups I, II, III, and IV, respectively, of 15, 13, 30, and 26 mg. per 100 gm. body weight.

Microscopic: Among the several histologic techniques used in this study, the most revealing was the periodic acid-fuchsin stain. The descriptions which follow, therefore, refer only to tissues stained by this method. Substances noted as periodic acid-fuchsin-positive appear as red or purple and are inferentially considered to be of glycoprotein nature.

Kidney: Controls. These animals had been uninephrectomized and maintained on 1% sodium chloride solution as drinking fluid during the course of the experiments. Nevertheless, the kidneys seemed substantially normal (Fig. 3 *A*); the periodic acid-fuchsin-positive structures were the basement membranes of glomeruli and tubules and the brush border of the proximal convoluted tubules. On close examination, small foci could be defined in some glomeruli in which the basement membrane, elsewhere clearly defined, appeared fuzzy and delaminated.

Kidney: Renin-treated. Although renin had been given for only three days, already definable glomerular abnormalities were present. The glomeruli (Fig. 3 *B*) seemed swollen and of increased cellularity; this appearance resulted in part from

hypertrophy of epithelial cells. The basement membranes were here and there delaminated, fragmented, or thickened with accumulations of amorphous purple material. Similar deposits appeared as cytoplasmic droplets in epithelial cells. The tubular structures were of normal appearance.

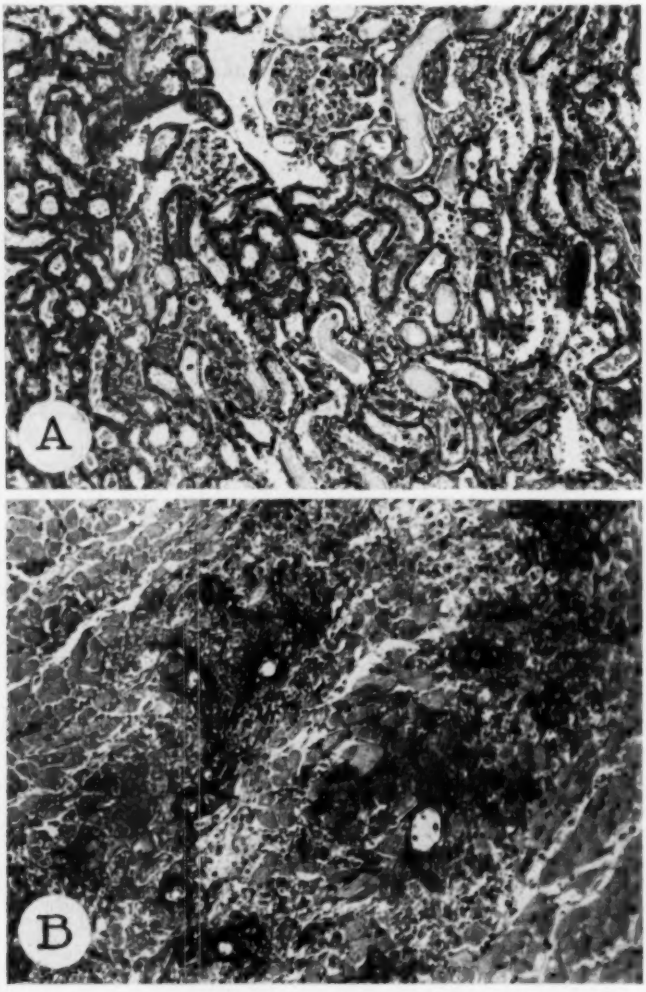

Fig. 2.—*A*, trichrome stain of kidney of cortisone-renin-treated rat showing large abnormal and a smaller normal glomerulus and hyaline and granular tubular casts; × 110. *B*, periodic acid-fuchsin stain of heart of cortisone-renin-treated rat showing vasodilation, degenerative vascular lesions, and perivascular deposits of periodic acid-fuchsin-positive material with foci of diffuse hemorrhages; × 140.

Kidney: Cortisone-treated. Lesions were more obvious in these than in the two preceding groups. They were again limited to the glomeruli, with the exception of granular deposits in some tubules. The glomeruli showed dense intercapillary and

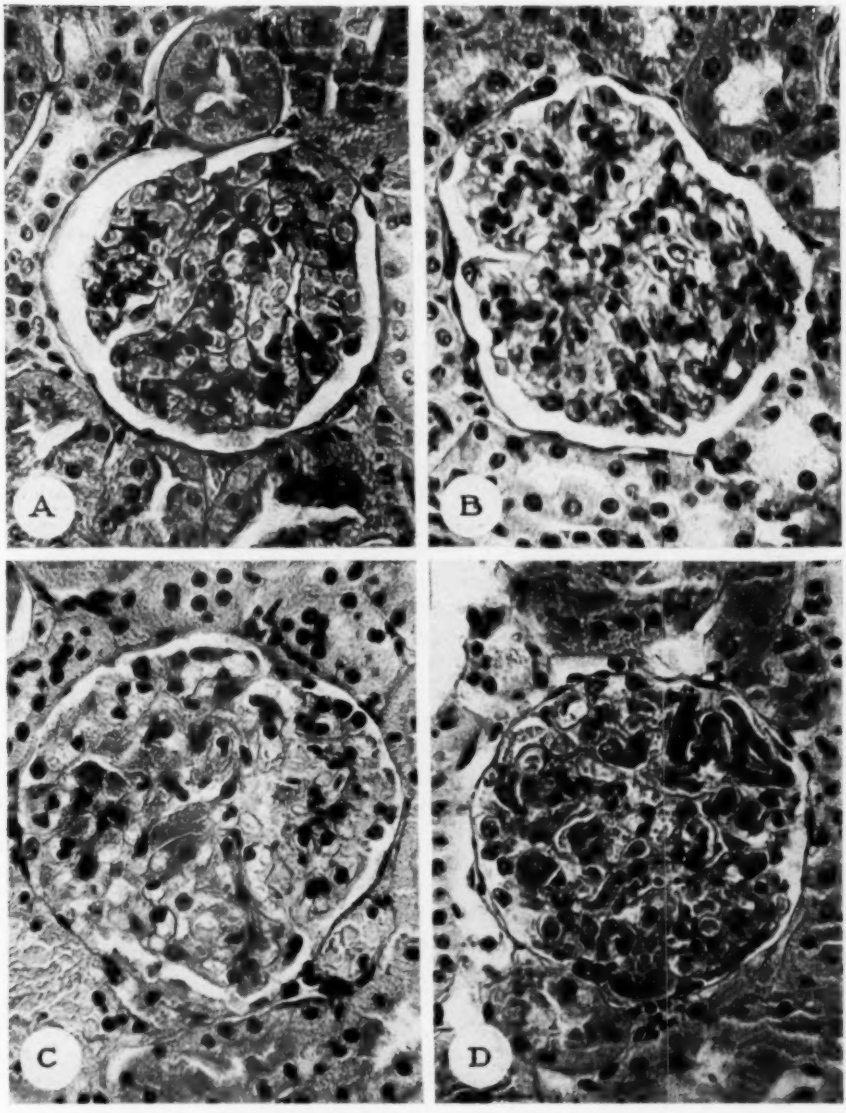

Fig. 3.—Periodic acid-fuchsin stains of representative glomeruli. *A*, control; *B*, renin-treated; *C*, cortisone-treated, and *D*, cortisone-renin-treated. All magnifications are × 550. Lesions are described in text.

intracellular accumulations of discrete granules of periodic acid-fuchsin-positive material. The basement membranes were diffusely split, swollen, and, in other sites, atrophic and fragmented (Fig. 3 *C*). The epithelial cells were hypertrophic; the swollen glomerulus nearly filled the capsular space.

Kidney: Cortisone and renin-treated. Lesions in this group were severe enough to be demonstrable on gross inspection. Areas of focal glomerular necrosis and/or thrombosis were visible under low-power magnification and were in close association with tubules showing degenerative changes and containing both granular (periodic acid-fuchsin-negative) and hyaline (periodic acid-fuchsin-positive) casts. The tubules were here and there dilated, and the tubular epithelium was atrophic or regenerative in varying degree (Fig. 2 *A*). Red cells were present in some few tubules; however, the kidneys did not show the diffuse hemorrhages or the pigment casts noted in desoxycorticosterone-renin disease.[2] In contrast with this condition also, the arterioles were more severely damaged, with thrombosis of some and hyalinization of the vessel wall; however, these changes did not provoke perivascular reaction (Fig. 4 *A*).

At high magnifications, the lesions seemed to be basically exaggerations of the sequence of degenerative changes described in the groups above. They characteristically varied widely in severity and in expression within the same section and even within the same field, so that even some of the most severe changes occurred in tissues which also contained substantially intact glomeruli and tubules.

The glomerular lesions consisted variously of (*a*) deposition of hyaline (periodic acid-fuchsin-positive) material, (*b*) capillary thrombosis, and (*c*) necrosis. Lesions associated with deposits (Fig. 4 *A*) seemed to result from the coalescence of granular red material into dense purple accumulations which invaded the mesangium and compressed the capillaries. This lesion was associated with intense focal disruption, swelling, delamination, atrophy, and even loss of the basement membrane. The associated capsular spaces often contained red droplets which seemed to be extrusions of this material; some of them contained a pink deposit, possibly representing dilution of the periodic acid-fuchsin-positive substance in a protein exudate. In the thrombotic lesions (Fig. 3 *D*), the mesangium was similarly filled with red droplets and granules, and the basement membranes were degenerated; the hyaline deposits were not as dense; the capillaries were wholly or partially filled with fibrillar thrombi, which could be seen to form on the endothelium. The glomeruli were swollen and tended to fuse with capsular epithelium. The necrotic lesions found in some of the animals are shown in Figure 4 *B*; there was complete loss of normal structure in all or part of a tuft, sometimes associated with polymorphonuclear invasion.

The parietal basement membrane showed changes similar to those observed in the visceral basement membrane, namely, swelling, delamination, atrophy, and dissolution. Parietal, like visceral, epithelial cells were swollen and hypertrophic but not proliferative.

The proximal tubules, particularly those associated with damaged glomeruli, contained hyaline red droplets in their lumina and similar material in the cells (Fig. 4 *C*); this was associated with loss of the brush border. The process seemed to be one of athrocytosis of a glycoprotein released from the glomeruli. What seemed to be the distal tubules of these injured nephrons often contained hyaline red casts,

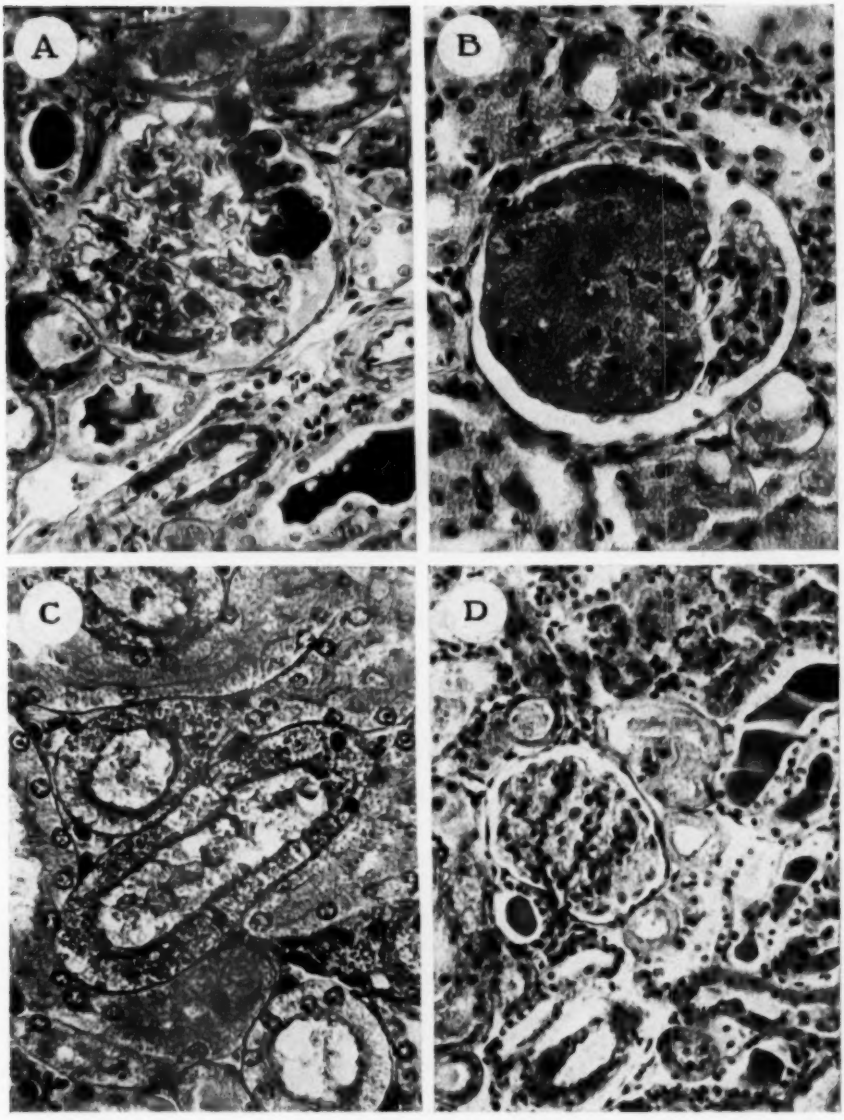

Fig. 4.—Periodic acid-fuchsin stains of kidneys from cortisone-renin-treated rats. *A*, deposit type of lesion in glomerulus; hyaline casts in tubules and focal hyaline deposits in arteriole; × 400. *B*, necrotic type of glomerular lesions; × 650. *C*, proximal tubule adjacent to an affected glomerulus showing intratubular and intracellular droplets of hyaline material, disruption of brush border, and disruption of basement membrane; × 540. *D*, necrotic tubules and hyaline tubular casts; × 270.

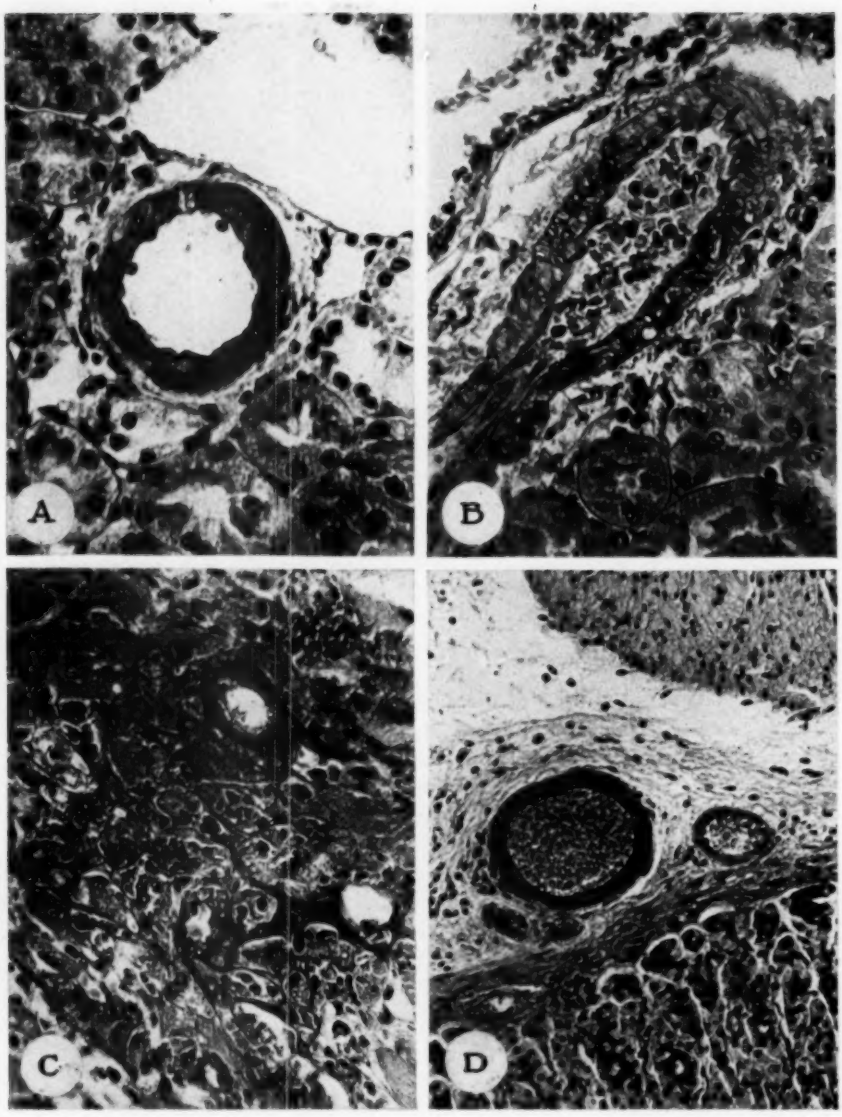

Fig. 5.—Periodic acid-fuchsin stains. *A*, complete hyalinization of renal arteriole; × 620. *B*, early focal intercellular hyaline films in renal arteriole; × 500. *C*, heart showing dilated, infiltrated small vessels with swollen discontinuous endothelium and perivascular hyaline deposits with loss of normal muscle cells; × 450. *D*, hyalinized dilated arteriole in stomach wall; × 270.

apparently as a result of concentration and precipitation of nonathrocytosed material. The association of hyaline athrocytosis with loss of the brush border suggests that the process was injurious to the cell; as such, although no details could be made out, it may represent the first stage of a sequence which ends in the necrosis observed in some of the tubules (Fig. 4 *D*). A glomerular origin of the hyaline droplets and casts was confirmed by the absence of these in tubules associated with a necrosed glomerular tuft. The subepithelial basement membranes of affected tubules were fragmented, frayed, swollen, and sometimes disrupted with an overflow of the epithelial cells into the interstitial spaces.

The arteriolar lesions were severest in animals manifesting glomerular necrosis; in comparison with the lesions observed in rats made hypertensive by other means, the lesions were remarkably intense. Both large and small arterioles showed patchy foci of degeneration; these seemed to begin as an infiltration of films of periodic acid-fuchsin-positive material between the muscle cells of the media (Fig. 5 *B*) which, as it extended, replaced all the normal constituents of the media (Fig. 5*A*). Some of the affected small arterioles, such as afferent arterioles, were thrombosed, and the associated glomeruli were either thrombosed or necrotic.

Heart: Myocardial lesions occurred only in the cortisone plus renin group. These consisted either of hemorrhage, with resultant loss of tissue, or of focal infiltrations of the interstitium with periodic acid-fuchsin-positive hyaline substance similar in appearance to that found in the kidney (Fig. 2 *B*). These deposits seemed to spread out from dilated capillaries, around which the deposits were arranged. The walls of these vessels were irregularly thickened with the hyaline material, so that the lumina were eccentric, and the endothelium seemed here and there to have lost its continuity (Fig. 5 *C*). As the interstitial deposits spread around muscle cells, the latter—possibly because of nutritional interference—showed cytoplasmic vacuolization and nuclear pyknosis. In contrast, nuclei of interstitial cells seemed normal, even though the cells themselves were lost in a mass of hyaline deposit.

The arterioles, in contrast to the capillaries, showed focal areas of hyaline deposition in their media but no tendency of the deposit to spread beyond the vessel wall.

Other Tissues: These, like myocardial lesions, were observed only in the cortisone-renin series; they occurred in the intestinal tract, pancreas, and mesentery. The intestinal lesions consisted of edema and areas of mucosal necrosis usually adjacent to dilated hyalinized arteriolar vessels (Fig. 5 *D*). Such areas were often hemorrhagic and usually infiltrated with red hyaline deposits, the precise distribution of which was uncertain because of the associated necrosis and loss of normal structure. Pancreatic and mesenteric arterioles showed similar hyalinization and dilatation. In contrast with renal or desoxycorticosterone hypertensive disease, these splanchnic vascular lesions were not associated with intravascular or perivascular cellular infiltrates.

COMMENT

The lesions elicited by giving renin to rats pretreated with cortisone and salt show that acute vascular diseases can result from abnormalities which are metabolic, rather than immunologic or infectious. Some of the lesions, notably the lesions of

the glomeruli, have a great deal in common with those observed in some forms of experimental and clinical glomerulonephritis [5] and in experimental renal hypertension,[6] and they demonstrate the tendency of a tissue to show a narrow range of response to widely disparate stimuli. These changes, like those observed in rats pretreated with desoxycorticosterone, recall the etiologic associations of eclamptogenic toxemia of pregnancy with sodium retention and renal damage and the therapeutic associations of sodium restriction in acute and chronic glomerulonephritis in some forms of essential hypertension and during treatment with cortisone and corticotropin (ACTH). A further clinical association is the observation that, while cortisone halts the extrarenal manifestation of systemic lupus erythematosus, it rarely remits and sometimes intensifies the progress of the associated renal diseases.[7]

From a technical aspect, our observations support the view that the periodic acid-fuchsin staining procedure is advantageous in the study of clinical [8] or experimental [9] renal lesions. While the trichrome method reveals the hyaline deposits, only the periodic acid-fuchsin staining procedure permits the demonstration that these seem to depend on abnormalities of basement membranes.

More fundamentally, the observations reaffirm the central position of the sodium ion in the genesis of vascular lesions. Association of sodium excess and vascular damage has been demonstrated in dogs. Administration of salt and renin to nephrectomized dogs [10] or even of a balanced electrolyte solution alone [11] can elicit severe vascular damage with hypertension; a similar situation seems to prevail in nephrectomized dogs maintained alive for long periods by some form of dialysis.[12] All these varied acute vascular lesions, like those which can be precipitated by giving renin

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6. Toussaint, C.: Les modifications pathologiques provoquées par l'ischémie partielle du rein, l'hydronéphrose bilatérale et la néphrectomie totale chez le rat intact et chez le rat surrénalectomisé, Rev. belge path. et méd. expér. **21**:465, 1952.

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8. McManus, J. F. A.: The Periodic Acid Routine Applied to the Kidney, Am. J. Path. **24**:643, 1948.

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11. Orbison, J. L.; Christian, C. L., and Peters, E.: Studies on Experimental Hypertension and Cardiovascular Disease: Method for the Rapid Production of Malignant Hypertension in Bilaterally Nephrectomized Dogs, A. M. A. Arch. Path. **54**:185, 1952.

12. Grollman, A.: Experimental Studies on Hypertension, in Hypertension: A Symposium, edited by E. T. Bell, Minneapolis, University of Minnesota Press, 1951. Kolff, W. J., and Fisher, E. R.: Pathologic Changes After Bilateral Nephrectomy in Dogs and Rats, Lab. Invest. **1**:351, 1952. Leonards, J. R., and Heisler, C. R.: Blood Pressure in Bilaterally Nephrectomized Dogs, Federation Proc. **11**:247, 1952.

intravenously to nephrectomized or renally insufficient dogs and rabbits,[13] have in common the factors of arterial hypertension and enhanced susceptibility of the nephrectomized animals to the angiotoxic and pressor effects of renin.

The role of sodium is also demonstrated in rats in which pretreatment with isotonic saline and desoxycorticosterone followed by administration of renin results in acute renal and generalized vascular disease associated with hypertension; similar but somewhat milder symptoms are also obtained in rats given hypertonic saline and then given injections of renin.[14] In these animals, renal insufficiency, as evidenced by fluid retention, anuria, or oliguria, and decrease in urea clearance, constitutes an important part of the syndrome.[1] It is therefore possible that in these rats we are reproducing conditions similar to those obtained by nephrectomy, renin, and saline, which in dogs elicit hypertensive vascular disease.[10]

The importance of sodium is further demonstrated by the following observation. During the course of the experiments described in the present paper, one group of rats maintained on 1% saline and cortisone were inadvertently given distilled water two days before administration of renin. Under these conditions, renin did not elicit fluid retention, oliguria, or the morphologic changes observed in rats receiving salt, cortisone, and renin. Thus, the angiotoxic effects of renin are integrally associated with sodium repletion.

The increased sensitivity to renin in rats and dogs suggests that increased arterial pressure as such is one of the factors in the genesis of acute vascular disease. This is supported by the observation of Gaunt,[15] who has shown that treatment of desoxycorticosterone-treated rats with a depressor drug, hydralazine hydrochloride (Apresoline), at the time of administration of renin prevents the acute eclampsia syndrome and vascular disease.

However, the level of arterial tension does not seem to be the only conditioning factor, since animals with normal function and relatively normal distribution of electrolytes can long tolerate high levels of arterial pressure.[16] The metabolism of sodium ion is admittedly severely altered by both desoxycorticosterone and cortisone. These steroids act on it in different ways: The action of desoxycorticosterone is primarily renal, while that of cortisone is believed to be primarily on the distribution of sodium between intracellular and extracellular fluids.[17] However, both

13. (*a*) Winternitz, M. C.; Mylon, E.; Waters, L. L., and Katzenstein, R.: Studies on the Relation of the Kidney to Cardiovascular Disease, Yale J. Biol. & Med. **12**:623, 1940. (*b*) Leiter, L., and Eichelberger, L.: Pressor Kidney Extract ("Renin") and the Production of Cardiac and Gastro-Intestinal Hemorrhages and Necroses in Dogs with Abnormal Renal Circulation, J. Mt. Sinai Hosp. **8**:744, 1942. (*c*) Pugh, R. C. B.; Pickering, G. W., and Blacket, R. B.: The Production of Vascular Lesions and Cardiac Hypertrophy by Infusions of Renin and Noradrenaline in the Rabbit, Clin. Sc. **11**:241, 1952.

14. Footnotes 1 and 2.

15. Renzi, A. A., and Gaunt, R.: Effect of Apresoline (1-Hydrazinophthalazine) on the Experimental "Eclampsia-Like" Syndrome and on Related Aspects of Water Metabolism, Federation Proc. **12**:360, 1953.

16. Goldblatt, H.: The Renal Origin of Hypertension, Publication 14, American Lecture Series, Springfield, Charles C Thomas, Publisher, 1948. Pugh, Pickering, and Blacket.[13c]

17. Overman, R. R.; Davis, A. K.; Bass, A. C.; Hudson, V. C., and Stevenson, E. A.: Effects of Cortisone and DCA on Radiosodium Transport in Normal and Adrenalectomized Dogs, Am. J. Physiol. **167**:333, 1951.

elicit hypertension; they differ in that the hypertension caused by cortisone is of rapid onset, moderate in degree, and independent of dietary sodium,[18] while that caused by desoxycorticosterone is delayed, progressive, severe, and sodium-dependent.[19] The ultimate nature of the hypertension in each case is not known, but it can be assumed to reflect changes in the state of the vascular musculature related to some maldistribution of sodium ion. This metabolic change in vessels which results in increased arterial pressure is also associated with hyaline infiltration and damage to nephrons and blood vessels.[20] Indeed, while the initial lesions occur in glomerular membranes which are exposed to pressure, ultimately lesions also appear not only in arteriolar but also in peritubular membranes and brush borders.

The description above indicates and accords with the view [9] that the basic lesion is a degeneration of epithelial basement membranes in nephrons and of vascular membranes. Hyaline deposits probably result from the combination of depolymerized carbohydrate components, with proteins seeping through from the plasma.[9] High-salt diet plus uninephrectomy associated with treatment with cortisone, desoxycorticosterone, or renin can initiate these morphologic alterations which are intensified by combined treatment with both steroids [9] or with one steroid plus renin. That the degenerative lesions elicited by desoxycorticosterone (or cortisone) plus renin are more acute and severer than those following cortisone plus desoxycorticosterone suggests a synergistic effect of renin, since the latter alone is less damaging than either of the two steroids. The deposits of hyaline which eventually form result in cell necrosis and inflammatory reaction; they cause loss of the brush border, death of the cells, and, in the desoxycorticosterone-treated and renal hypertensive rats, interstitial and perivascular inflammatory reactions. Periarteritis does not appear with cortisone treatment, because one of its basic activities is the suppression of mesenchymal proliferative activity and inflammatory reaction.[19a]

Thus, the concept of the metabolic vascular disease described above and of that caused by desoxycorticosterone plus renin is that it stems from the effects of changes in the distribution of sodium ion which elicit alterations of basement membranes and like structures and changes in cardiac and arteriolar function which result in hypertension; the effect of hypertension on the sensitized vessels, then, determines the speed of onset of vascular lesions.

This concept leaves unanswered two problems: One is the nature of the difference between the syndrome elicited by cortisone plus renin and by desoxycorticosterone plus renin; the other is the specificity of renin in each of these. As to the difference between the syndromes, the most significant is the relative absence of a hemorrhagic diathesis in cortisone-renin disease and the severity of this in desoxycorticosterone-renin disease; thus, the anuria so common in desoxycorticosterone-renin disease, which probably results from the superimposition of hemoglobinuric

18. Knowlton, A. I.; Loeb, E. N.; Stoerk, H. C.; White, J. P., and Heffernan, J. F.: Induction of Arterial Hypertension in Normal and Adrenalectomized Rats Given Cortisone Acetate, J. Exper. Med. **96**:187, 1952.

19. (*a*) Masson, G. M. C.; Corcoran, A. C.; Dustan, H., and Page, I. H.: Cardiovascular and Metabolic Effects of Cortisone, Hydrocortisone and Other Steroids in the Rats, Am. J. M. Sc. **224**:175, 1952. (*b*) Selye, H.; Stone, H.; Timiras, P. S., and Schaffenburg, C.: Influence of Sodium Chloride upon the Actions of Desoxycorticosterone Acetate, Am. Heart J. **37**:1009, 1949.

20. Toussaint, C.: Effets de la cortisone (17-hydroxy-11-déhydrocorticostérone) sur le rein et l'appareil cardiovasculaire du rat, Compt. rend. Soc. biol. **145**:1427, 1951.

nephrosis on the basic renal lesion, contrasts with the relative oliguria seen with cortisone-renin. With this exception and the predictable difference in mesenchymal proliferative changes, the syndromes and lesions are basically the same, with only the quantitative difference that the arteriolar hyaline lesions of cortisone-renin disease are more severe.

No direct answer can be given as to the specificity of renin and its product, angiotonin, in this syndrome. Under the conditions of the experiment it is given subcutaneously and causes a prolonged increase in arterial pressure which in rats given cortisone superimposes on the moderate-existing hypertension. The possibility has not been excluded that other pressor agents of prolonged action might precipitate a similar condition. However, renin is known to have other than pressor and vasoconstrictor activities; renin (or angiotonin) also initiates increased urinary outputs of water, sodium, chloride, and protein [21]; the latter effect is attributed to increased glomerular permeability (of which the changes noted above in renin-treated rats may be the structural expression) with decreased proximal tubular athrocytosis.[22] These membrane effects of renin suggest that it has metabolic, as well as pressor, effects on blood vessels. In accord with this is the hypertrophy of the zona glomerulosa of the adrenals seen in renal hypertensive rats or in rats chronically treated with renin,[23] as well as other observations which lead to the concept of renal-adrenal interplay in experimental renal hypertension.[24] Thus it seems likely that the effect of renin in the cortisone-renin or in the desoxycorticosterone-renin syndrome extends beyond its effect alone on arterial pressure.

SUMMARY

Administration of a renin-containing renal extract to uninephrectomized rats given saline to drink and pretreated with cortisone elicits a syndrome consisting of acute water retention, relative oliguria, hypertension, and death; it is accompanied by acute renal and vascular lesions. This syndrome resembles that produced under similar conditions by pretreatment with desoxycorticosterone instead of cortisone; it differs, however, by the almost complete absence of hemorrhages and by an increased incidence of vascular lesions.

The basic lesion is a degeneration of membranes with resulting increase in permeability and formation of hyaline deposits. In the arterioles hyaline degeneration is limited to the vessel wall, while in the blood vessels of capillary size the hyaline material spreads out into the surrounding tissues which become necrotic. The glomerular lesions are similar to those seen in experimental renal hypertension and in some forms of clinical and experimental membranous glomerulonephritis.

Mrs. E. Haskill gave her technical assistance in the preparation of this paper, and Mr. T. Lannon provided the photomicrographs.

21. Hughes-Jones, N. C.; Pickering, G. W.; Scarborough, L. H., and Vanderbrouke, J. J.: The Nature of the Action of Renin and Hypertension of Renal Function in the Rabbit, J. Physiol. **109**:288, 1949.

22. Lippman, R. W.; Ureen, H. J., and Oliver, J.: Mechanism of Proteinuria: Effect of Renin on Hemoglobin Excretion, J. Exper. Med. **93**:605, 1951.

23. Deane, H. W., and Masson, G. M. C.: Adrenal Cortical Changes in Rats with Various Types of Experimental Hypertension, J. Clin. Endocrinol. **11**:193, 1951.

24. Masson, G. M. C.; Corcoran, A. C., and Page, I. H.: Nouveau concept sur la pathogénèse de l'hypertension expérimentale d'origine rénale in Congrès mondial de cardiologie, Vol. 3, Paris, J. B. Baillière et fils, 1952.

RETARDATION OF ATHEROMATOSIS AND ADRENAL ENLARGEMENT BY HEPARIN IN THE RABBIT

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WHILE it is generally accepted that the etiology of human atherosclerosis is a complex phenomenon involving several factors, according to some authors (Gubner and Ungerleider,[1] Gofman and others [2]), derangements of lipid metabolism play an important pathogenic role.

In recent years heparin has been shown to affect certain phases of lipid metabolism, although the mechanism of its action is as yet obscure. Thus, Chargaff [3] claimed that heparin splits lipoproteins in vitro, releasing the lipid component. Hahn [4] reported that heparin abolishes the lipemia following fat meals. His results were confirmed by Weld,[5] Waldron and Friedman,[6] and Anderson and Fawcett.[7] Finally, Faber [8] claimed that extracellular cholesterol deposits always occur in metachromatic tissue areas, that is, in areas which might contain heparin-like compounds.

In view of some of these data it appeared of interest to study the effect of heparin on the atheromatous process produced by cholesterol feeding in the rabbit.[9] Furthermore, since Parrot and Laborde [10] demonstrated that heparin binds histamine, the effect of an antihistaminic agent on cholesterol-induced atheromatosis was also studied.

While this work was in progress, Graham and others [11] reported without data that intravenous heparin injections, initiated at the start of cholesterol feeding,

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From the Department of Anatomy, University of British Columbia.

1. Gubner, R., and H. E. Ungerleider: Am. J. Med. (No. 1) **6**:60, 1949.
2. Gofman, J. W., and others: Circulation (No. 2) **2**:161, 1950.
3. Chargaff, E.: J. Biol. Chem. **142**:491, 1942.
4. Hahn, P. F.: Science **98**:19, 1943.
5. Weld, C. B.: Canad. M. A. J. **51**:578, 1944.
6. Waldron, J. M., and Friedman, M. H.: Fed. Proc. **7**:130, 1948.
7. Anderson, N. G., and Fawcett, B.: Proc. Soc. Exper. Biol. & Med. **74**:768, 1950.
8. Faber, M.: Arch. Path. **48**:342, 1949.
9. A preliminary communication of the results of this study was made by Dr. Constantinides at the Sixth Session of the Western Regional Group, Medical Research Division, National Research Council of Canada, Feb. 8, 1952.
10. Parrot, J. L., and Laborde, C.: Compt. rend. Soc. biol. **145**:1047, 1951.

retarded the development of atheromatosis in rabbits. The morphological data of the present paper, obtained with a different experimental arrangement, are in full agreement with and expand the claim of Graham and others.

MATERIALS AND METHODS

Three similar experiments of different duration were carried out, employing a total of 30 rabbits.

EXPERIMENT 1 (five and one-half weeks duration).—Nine albino rabbits of an average initial body weight of 2 kg. were divided into three groups, each consisting of one female and two male rabbits. All animals were kept on tap water and rabbit chow ad libitum and had free access to suitable greens daily.

The animals of Group I served as untreated controls, while those of Groups II and III were force-fed (by stomach tube) 0.9 gm. cholesterol (Eastman-Kodak) daily over a period of five and one-half weeks. The daily cholesterol dose was dissolved in 12 ml. Mazola oil. In addition to being fed with cholesterol oil, each animal of group III received subcutaneous injections of 10 mg. (1,000 units) heparin (Connaught) twice daily throughout the duration of the experiment.

At the end of the cholesterol treatment period, all animals were killed by a blow on the neck and were autopsied.

Each aortic tube was slit longitudinally from the iliac bifurcation to the heart, and the atheromatosis was graded macroscopically as follows: 0, no lesions; +, slight atheromatosis; ++, moderately severe atheromatosis; +++, severe atheromatosis; and ++++, maximum atheromatosis, i. e., confluent plaques lining the entire tube.

Samples of the heart, the liver, and the adrenal gland were removed for histological examination. The fixed weight of the left adrenal gland was obtained in every animal.

The turbidity of the serum was graded macroscopically in blood samples obtained from all animals just prior to sacrifice on the basis of the following scale: 0, completely clear serum; +, slightly cloudy serum; ++, moderately cloudy serum; +++, completely opalescent, milk-white serum. In the same samples, total serum cholesterol was estimated chemically with the Sperry [12] technique. The specifications of the Committee on Cholesterol Methods of the American Society for the Study of Arteriosclerosis (annual meeting of Nov. 1, 1948) were strictly observed.

Death was timed to occur four hours after the last cholesterol feeding and (or) heparin injection.

EXPERIMENT 2 (seven and one-half weeks' duration).—Seven female albino rabbits of an average initial body weight of 1.5 kg. were divided into three groups as before: Group I consisting of two rabbits, Group II of three rabbits, and Group III of two rabbits. The experimental procedure was identical with that employed in the previous experiment, but cholesterol oil was given for seven and one-half weeks, and heparin injections were given only during the latter four weeks of cholesterol treatment.

At the end of the cholesterol treatment period, all animals were killed with ether and were autopsied. Aortic atheromatosis and serum turbidity were graded, while total serum cholesterol was estimated chemically as before.

Samples of the aorta, heart, liver, kidney, adrenal gland, thymus, and brain were removed for detailed histological examination. The thymus and a kidney sample were fixed in a formalin-acetic acid-alcohol mixture. The remaining organs and other kidney sample were fixed in formalin. The following four methods were employed in the histological examination of the aorta: (*a*) lipids with Sudan black in frozen sections, (*b*) elastic tissue with orcein in paraffin sections, (*c*) hemalum-phloxine-saffron in paraffin sections, and (*d*) toluidine blue in paraffin sections. Frozen sections from the liver and the adrenal gland were stained with Sudan black. Paraffin sections from the heart, the brain, and a kidney sample were stained with hemalum-phloxine-saffron. Paraffin sections from another kidney sample and from the thymus were stained

12. Sperry, W. M.: Am. J. Clin. Path. **2**:91, 1938.

with toluidine blue. All paraffin sections were cut at 7 μ. The frozen sections of the aorta were cut at 20 μ, those of the liver at 25 μ, and those of the adrenal gland at 40 μ.

EXPERIMENT 3 (nine and one-half weeks' duration).—Fourteen female albino rabbits of an average initial body weight of approximately 2 kg. were divided into four groups. Groups I and IV consisted of three rabbits, Groups II and III of four rabbits each.

Summary of Data

Group	Rabbit No.	Aortic Atheromatosis (Graded)*	Serum Turbidity (Graded)†	Serum Cholesterol, Mg./100 Cc. Serum	Adrenal Weight, Mg./Kg. Terminal Body Weight	Initial Body Weight, Kg.	Terminal Body Weight, Kg.
		Experiment 1.—Duration 5½ Weeks					
Controls	1	0	0	83	28	1.8	3.1
	2	0	0	46	34	1.8	3.2
	3	0	0	70	30	2.2	3.0
Cholesterol	4	+	+++	1,280	133	1.5	3.0
	5	+	0	...	150	2.7	3.4
	6	+	+++	1,062	155	2.4	3.7
Cholesterol plus Heparin	7	0	0	448	88	2.2	3.0
	8	0	+	714	92	2.2	3.7
	9	0	+	797	72	1.7	3.2
		Experiment 2.—Duration 7½ Weeks					
Controls	1	0	0	75	38	1.5	2.9
	2	0	0	75	27	1.5	3.2
Cholesterol	3	+++	+++	1,580	96	1.5	2.6
	4	++	+++	1,680	122	1.5	2.6
	5	++++	+++	1,420	101	1.5	3.0
Cholesterol plus Heparin	6	0	0	720	65	1.5	2.5
	7	0	0	850	77	1.5	2.6
		Experiment 3.—Duration 9½ Weeks					
Controls	1	0	0	..	34	1.9	3.0
	2	0	0	..	28	1.8	2.7
	3	0	0	..	39	2.9	4.5
Cholesterol	4	+	+	..	95	2.2	3.2
	5	++++	+	..	295	1.9	2.9
	6	++	++	..	132	2.2	3.6
	7	+	+++	..	177	2.0	3.2
Cholesterol plus Heparin	8	0	0	..	137	2.2	3.4
	9	+	++	..	225	2.0	2.7
	10	+	0	..	135	2.1	3.1
	11	0	0	..	104	2.0	3.0
Cholesterol plus Phenothiazine (Phenergan)	12	++	+++	..	263	2.1	3.0
	13	+	+	..	181	2.7	4.0
	14	+++	+++	..	307	1.9	2.9

* Key to symbols: 0, no lesions; +, slight atheromatosis; ++, moderately severe atheromatosis; +++, severe atheromatosis; ++++, maximum atheromatosis, i. e., confluent plaques lining the entire tube.

† Key to symbols: 0, completely clear serum; +, slightly cloudy serum; ++, moderately cloudy serum; +++, completely opalescent, milk-white serum.

The animals of Group I served as untreated controls, while those of Groups II, III, and IV were force-fed cholesterol oil for nine and one-half weeks. During the last five weeks of the cholesterol treatment period, each animal of Group III received heparin as before, while each animal of Group IV received one daily subcutaneous injection of 12.5 mg. of phenothiazine, (Phenergan, Poulenc), a potent antihistaminic agent.[13]

13. Documents expérimentaux et cliniques sur le phenergan, Ed. 4, Paris, Soc. Paris. Expans. Chimique, 1951.

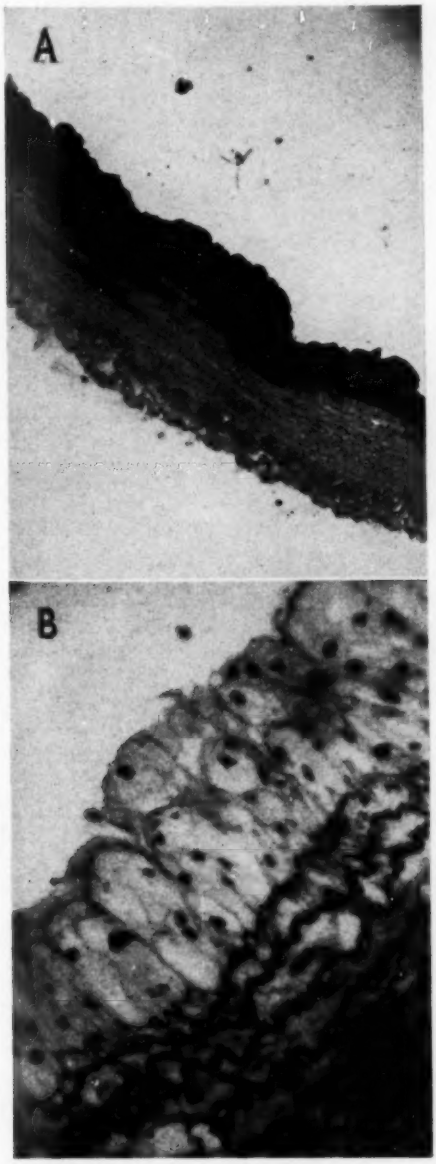

Fig. 1.—*A*, low-power; Sudan black. Aorta of a cholesterol-fed rabbit. Note the great accumulation of lipids in the intima and the inner third of the media. *B*, high-power; elastic stain. Intima and subjacent media of the atheromatous aorta of a cholesterol-fed rabbit. Note the thickening of the intima which consists of lipid-laden cells. Also note that as the atheromatous process penetrates into the underlying media, its elastic layers are broken up.

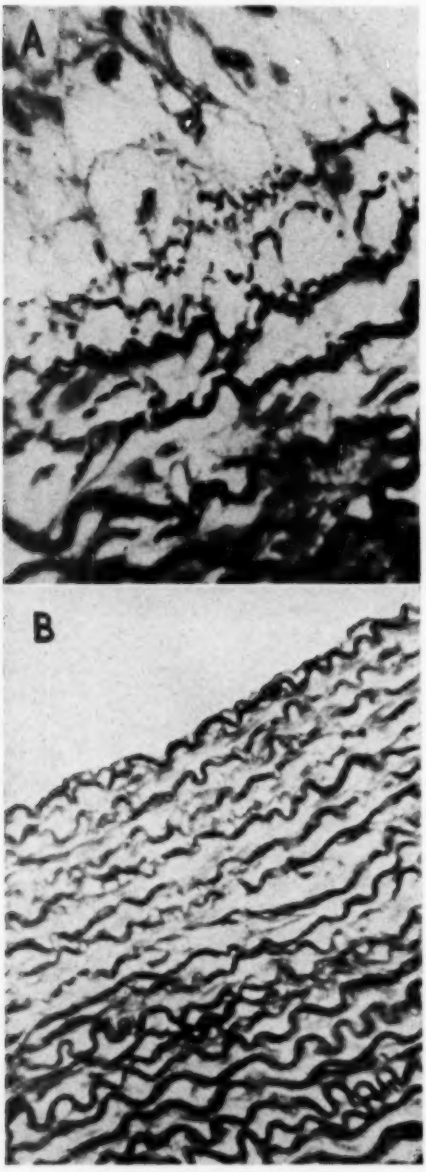

Fig. 2.—*A*, oil immersion; elastic stain. Boundary between atheromatous intima and media in the aorta of a cholesterol-fed rabbit. Note the disintegration of the innermost elastic layers. *B*, high-power; elastic stain. Aorta of a cholesterol-heparin-treated rabbit. Note that the intima is normal (extremely thin) and the media-elastic layers are intact. Compare with Figure 1 *B*.

At the end of the cholesterol treatment period all animals were killed with ether and were autopsied. The end-points of this experiment were the same as in Experiment 1, except for serum cholesterol, which was not estimated.

RESULTS

Aortae.—In all three experiments, the rabbits of the cholesterol-treated group developed variable atheromatosis of the aorta in the form of macroscopically visible yellow plaques (Table). The lesions were particularly marked around the ostia of origin of the coronary, innominate, and thoracic arteries and at bifurcations. Figure 1*A* shows a representative frozen section from the aorta of a cholesterol-fed rabbit.

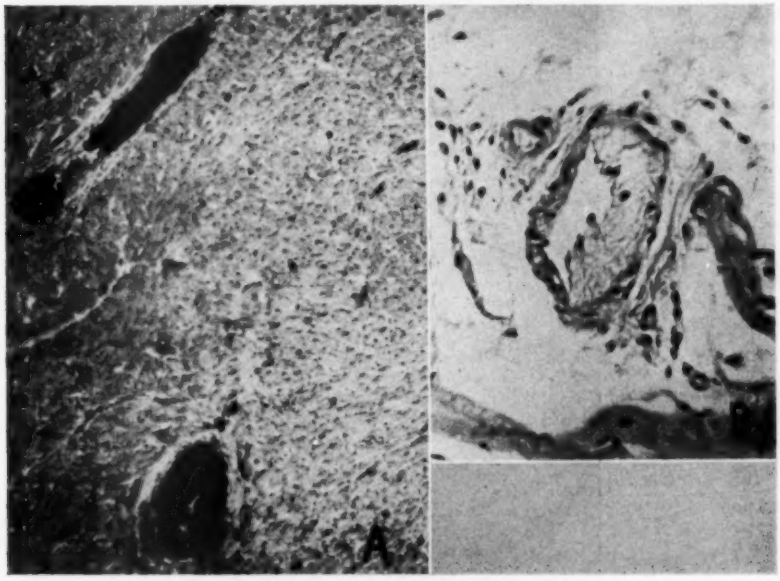

Fig. 3.—*A*, low-power; Sudan black. Atheromatous intramural coronary vessels from a cholesterol-treated rabbit. One vessel appears in longitudinal, the other in cross section. Note the almost complete occlusion of the lumen in both vessels. *B*, high-power; hemotoxylin and eosin stain. A small coronary vessel from a cholesterol-fed rabbit. Note the substantial atheroma occupying more than half of the right side of the wall and the narrowing of the lumen. Also note the destruction of the media in the atheromatous side of the vessel.

Heparin prevented the atheromatosis in the animals which were given cholesterol for five and one-half and seven and one-half weeks (Experiments 1 and 2). This inhibiting effect of heparin was diminished when cholesterol alimentation was prolonged to nine and one-half weeks (Experiment 3).

Phenothiazine did not appear to affect atherogenesis.

No atheromatosis developed in any normal control rabbit.

Examination of paraffin sections revealed that most atheromata were not limited to the intima but penetrated deep into the underlying media, destroying several

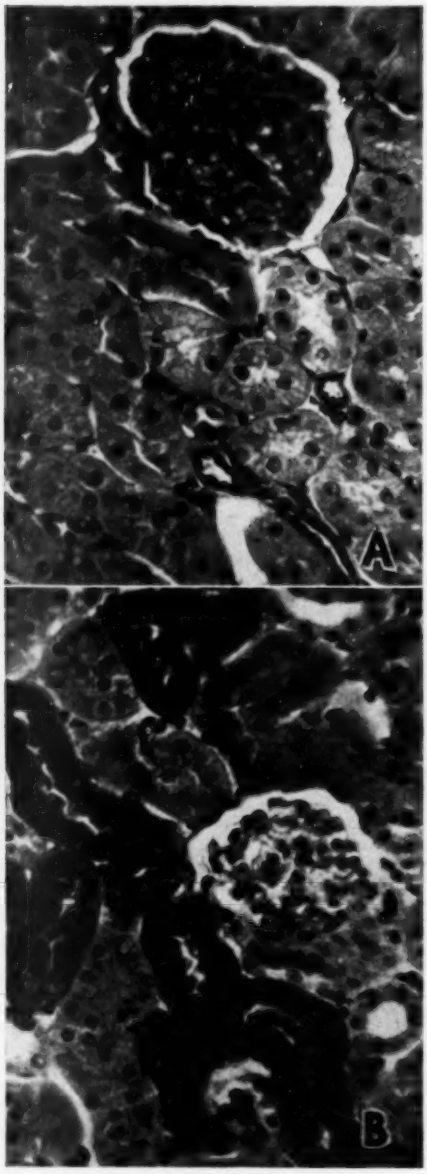

Fig. 4.—*A*, high-power; toluidine blue. Kidney of a cholesterol-fed rabbit. *B*, high-power; toluidine blue. Kidney of a cholesterol-heparin-treated rabbit. Note the appearance of intensely basophilic proximal tubules.

elastic layers on the way (Figs. 1*B* and 2*A*). The process of elastic tissue destruction usually commenced with a thinning or a cross-striation of the elastic tunics and progressed to their complete disintegration. Most of the lipid material of the atheromata was intracellular, i. e., it was included in the cells of a highly thickened intima. It appeared that the disintegration of the media-elastic tissue was secondary to the lipid infiltration. While none of the former process could be observed without the latter, the occasional atheroma that had not progressed beyond the intima was found to border on intact media-elastic layers. Consistent with the absence of lipid penetration, the elastic elements in the media of most cholesterol-heparin-treated rabbits were entirely normal (Fig. 2*B*).

Coronary Arteries.—Histological examination of the heart revealed that in most cholesterol-treated rabbits in Experiments 2 and 3 coronary atheromatosis developed, varying from partial to almost complete occlusion of several intramural coronary vessels (Fig. 3). In some cases this was accompanied by myocardial infarcts and scars.

None of the cholesterol-heparin-treated or control animals of any experiment showed such lesions.

Adrenals.—The adrenals of all cholesterol-treated rabbits were much larger (Table) and contained more lipids than those of the control animals. In some instances, the enlarged adrenal cortices displayed areas of cytolysis with extracellular deposits of a crystalline material which stained only feebly or not at all with Sudan black in frozen sections. The fact that these crystals were absent from paraffin sections would tend to identify them as lipid-soluble substances. Cytolysis and crystal formation were maximally manifest in the adrenals of the animals in which the highest degree of atheromatosis developed.

Heparin distinctly diminished the adrenal enlargement in the experiments which lasted five and one-half and seven and one-half weeks, but its effect was exhausted when cholesterol feeding was prolonged to nine and one-half weeks. It did not appear to influence the augmentation of the adrenal lipid content, but it diminished the cytolysis and the crystal formation. Phenothiazine had no effect on adrenal size or structure.

Serum Turbidity and Serum Cholesterol.—As can be seen in the Table, cholesterol oil feeding produced in almost all instances a marked lipemia and hypercholesteremia.

Heparin generally abolished or greatly diminished the lipemia, but it only partly inhibited the hypercholesteremia. Phenothiazine had no effect on lipemia.

Kidney.—No atheromatosis appeared in the renal vessels of any animal. Toluidine-blue-stained sections showed that the cells of numerous proximal convoluted tubules in the animals given heparin injections were packed with an intensely basophilic, slightly metachromatic material (Fig. 4). In hematoxylin and eosin-stained sections, the same tubules appeared to be strongly eosinophobic.

Thymus.—No abnormality was visible in any animal.

Brain.—This organ was examined only in Experiment 2. No atheromatosis was observed in the cerebral vessels as judged by histological examination, but variable lipoidosis appeared in the ependymal cells lining the choroid plexuses of the cholesterol- and cholesterol-heparin-treated rabbits.

Liver.—In all cholesterol-, cholesterol-heparin-, and cholesterol-phenothiazine-treated animals fatty change of the liver developed. There seemed to be no correlation between the degree of fatty change and the lipemia or atheromatosis. The livers of all normal controls were of normal gross and histological appearance.

Body Weights.—In all three experiments, heparin did not interfere with the growth of the animals (Table).

COMMENT

Considering the fact that heparin did not provoke any toxic symptoms and did not arrest the growth of the animals to which it was given, it seems certain that its atheromatosis-retarding properties represent a specific biological effect. It may be well to keep in mind that several "antiatherogenic" agents have been found to act by inducing emaciation, i. e., nonspecifically.[14]

While our phenothiazine experiment cannot be considered conclusive, it seems unlikely that heparin acted by blocking endogenous histamine, i. e., by decreasing intimal permeability. At present it appears reasonable to assume that the antiatherogenic effect of heparin is due to its antilipemic property.

The inhibition of the adrenal enlargement by heparin is, however, open to speculation. Although adrenal enlargement in cholesterol-treated rabbits has been recorded in the earliest relevant literature,[15] the role of the adrenals in the production of experimental atheromatosis has not been systematically studied and has remained obscure to date.[16] The adrenal reaction possibly represents a nonspecific response to the "stress" of lipemia (in the sense of Selye's "General Adaptation Syndrome" [17]), or, again, it may be specifically related to the high cholesterol intake of these animals. As it is, the relationship between cholesterol-induced atheromatosis, the adrenals, and heparin cannot be elucidated without separate investigations.

The decline of the antiatherogenic and adrenal-inhibiting action of heparin with the prolongation of cholesterol treatment is similarly difficult to explain. Future experiments will show whether it is possible to maintain these effects by progressively raising the heparin dosage or by employing other heparin-like compounds.

Finally, it can be assumed with reasonable certainty that the basophilic material which appeared in the renal tubules of the animals given heparin injections was heparin. This morphological finding is in line with the report of Wilander [18] and of Piper [19] concerning the fate of injected heparin in rabbits. The latter author found that 20 to 50% of an intravenous dose was excreted in the urine by glomerular filtration and tubular excretion.

In concluding these remarks it must be pointed out that it was not determined in this investigation whether the cholesterol solvent (mazola oil) contributed to the

14. Firstbrook, J. B.: Science **111**:31, 1950. Davidson, J. D.: Am. J. Med. (No. 6) **11**:736, 1951.

15. Fahr, T.: Verhandl. deutsch. path. Gesellsch. **15**:234, 1912.

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17. Selye, H.: Stress, Montreal, Acta Inc., Medical Publishers, 1950.

18. Wilander, O.: Scandinav. Arch. Physiol. (Supp. 15) **81**: 1938.

19. Piper, J.: Acta pharmacol. et toxicol. **3**:373, 1947.

development of atheromatosis. The term "cholesterol feeding" has therefore been used throughout this paper as equivalent to what should be more accurately called "cholesterol oil feeding."

Further experimentation on several issues raised by this study is in progress and will be reported shortly.

SUMMARY

Heparin administered subcutaneously and twice daily to rabbits force-fed cholesterol-oil for various periods of time had the following effects: It prevented the lipemia and reduced the hypercholesteremia; it greatly retarded the development of atheromatosis and adrenal enlargement; it did not prevent the accumulation of lipids in the liver or the adrenal.

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Histological slides were prepared by Miss Margaret McLean, and contributions to this study were made by Dr. Gerald W. Milton and Mr. John Cesar.

BREAST STROMA

Morphological and Histochemical Study

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INTRODUCTION

ALTHOUGH there is voluminous literature about the parenchyma of the breast and its diseases, relatively little attention has been given to the stroma. This lack of detailed information exists even though the stroma is the most prominent component in a common breast lesion, the fibroadenoma. The connective tissue of the breast is of particular interest because it is generally accepted that this stroma responds readily to certain hormones.

Connective tissues, in general, have been the subject of great interest in recent years,[1] as well as those diseases in which the connective tissue changes are especially prominent.[2] As a result of this interest, new techniques have been developed to study various aspects of connective tissue, and a considerable amount of new information has been gained.

Our interest in the connective tissue of the breast has been directed to two points of view: first, the changes as they are related to breast function and disease, and second, to the relationship between this tissue and other connective tissues. The material studied consisted of human breast tissue obtained from the files of a surgical pathology laboratory. This material has been studied with the help of some of the more recent techniques. The results of the study of this material have been compared with the information available in the literature. From this general orientation, a basis is obtained from which more specific problems related to both breast and connective tissue can be studied.

SUMMARY OF PERTINENT LITERATURE OF BREAST STROMA

In both rat [3] and human [4] embryos, the connective tissue of the developing breast structures can be distinguished from the surrounding mesenchyme. This tissue is

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From the Laboratory of the Barnard Free Skin and Cancer Hospital and the Department of Pathology, Washington University School of Medicine.

1. Connective Tissues: Transactions of the First (1950) Conference, edited by Ragan, C., New York, Josiah Macy Jr., Foundation, 1951. Connective Tissues: Transaction of the Second (1951) Conference, edited by Ragan, C., New York, Josiah Macy Jr., Foundation, 1952.
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first cellular and then becomes rich in fibers. Later, the connective tissue forms concentric rings around the epithelial elements. The degree of breast development in human newborn infants apparently varies greatly.[5] Geschickter [6] describes a rim of loose connective tissue surrounding the ducts in the secreting newborn breast; "active vascular stroma" was found. Later involutional changes included collagenous and hyalinized periductal connective tissue. In some patients Cheatle and Cutler [5] found "enormous" hyperplasia of stroma, sometimes associated with epithelial changes which they would have designated as "chronic mastitis" in the mature breast.

Some of the studies of changes during estrus or menstrual cycles have considered the stroma in more detail. The exact details of these breast changes have been debated for many years. One of the earlier studies was that of Rosenburg,[7] who studied a series of autopsy cases. He did not find that the stroma participated significantly in the breast changes associated with the menstrual cycle. Rather, he found that these changes consisted of complete regression of lobules in the postmenstrual and interval phases with subsequent development of lobules during the premenstrual phase. This report is not sufficiently illustrated to allow evaluation of the stromal changes in his material.

Dieckman [8] severely criticized the conclusions of Rosenburg on the basis of a study also made on autopsy material. He found that lobules persisted during all phases of the cycle. Epithelial changes were limited to the presence of cytoplasmic vacuoles in the basal cells of the epithelium during the premenstrual phase. On the other hand, the stroma was found to undergo a series of conspicuous cyclic changes. In the premenstrual phase the lobular stroma was moderately cellular and stood out because of the loosely woven net-like texture. Coincident with the loss of the loose structure of the premenstrual phase after menstruation, the stromal fibers became thickened and swollen; the basement membranes became wide and homogeneous. During the late postmenstrual phase collagen was present in the lobular stroma. In the early interval period the stroma was less cellular, whereas during the late interval phase the first signs of the next premenstrual phase could be detected. Dieckman considered the loose appearance of the stroma in the premenstrual phase to be due to edema. The vacuoles in the basal epithelial layer were thought to have a probable relationship to this edema. With regression of interstitial edema in the postmenstrual phase there was swelling of fibers until mature collagen was found in the later phases. Lobules were found in breasts of women with amenorrhea of several months' duration; a series of involutionary changes in these lobules was described. Dieckman considered lobule development to be a part of breast maturation which proceeded over a period of many years after puberty; menstrual changes were believed to be largely a matter of fluid shifts within the stroma.

5. Cheatle, G. L., and Cutler, M.: Tumors of the Breast: Their Pathology, Symptoms, Diagnosis and Treatment, London, Edward Arnold & Co., 1931.

6. Geshickter, C.: Diseases of the Breast: Diagnosis, Pathology, Treatment, Philadelphia, J. B. Lippincott Company, 1943.

7. Rosenburg, A.: Frankfurt. Ztschr. Path. **27**:466, 1922.

8. Dieckman, H.: Arch. path. Anat. **256**:321, 1925.

Numerous subsequent studies have generally confirmed the findings of Dieckman. Thus Moszkowicz [9] found similar menstrual changes in the stroma in lobules, in adenomas, in fibroadenomas, in cystadenomas, and in carcinomas. His study was made on surgically removed material. Moszkowicz states that the periductal and intralobular stromata are a functional unit. He believed this stroma to be incompletely differentiated as a result of a strong inhibitory influence exerted by the gonads. Dawson [10] found similar menstrual changes. Taylor [11] made similar observations on surgical material and suggested [12] a relationship between congestion and edema of the pelvic organs and the lobular edema of the breast, although insignificant evidence was presented.

Ingleby [13] found epithelial changes during the menstrual cycle similar to those described by Rosenburg; however, significant stromal changes were also found. Only ducts and fibrous tissue were found in the interval phase. In the premenstrual phase lobules form; the epithelial cells swell and form vacuoles; the lobular stroma undergoes "myxomatous or hyaline degeneration." Elsewhere [14] these stromal changes are described as "mucoid degeneration." The lobules undergo involution during the postmenstrual phase. Similar cyclic changes were found in fibroadenomas.

Cheatle and Cutler [5] review the conflicting reports on the breast changes associated with the menstrual cycle, although their position is not stated. However, they deal with the breast connective tissue in detail. In the concept of "mazoplasia," which these authors proposed, proliferation of pericanalicular and periacinous stromata with hyperplasia and desquamation of the epithelium of the terminal ducts are the prominent features. New ducts and acini may be formed in the process. In the excellent photographs the stroma has a loose cellular appearance with interstitial material. Mazoplasia is considered to be a physiological, rather than a pathological, state. Changes of mazoplasia are especially prominent during periods of breast activity. They are found in breasts of male and female infants; in women during pregnancy, puberty, and in the less active parts of the breast during lactation, and in painful enlarged breasts of men of the third and fourth decades. Fibroadenomas were the only pathological conditions found which resulted from mazoplasia. It is stated that fibroadenomas may arise from subepithelial, as well as pericanalicular and periacinous, connective tissue. Proliferation of elastic tissue occurred under certain conditions.

Geschickter [6] found that lobule formation does not occur in the breast until six or more months after the first menstrual period. With the development of ducts and lobules during adolescence, a pale-staining and vascular periductal zone of connective tissue is formed. He found that breasts were larger in the premenstrual than in the postmenstrual period by volumetric measurement. Two stages were distinguished during the menstrual cycle: a regressive stage from approximately the onset of menstruation until a few days after cessation of menstruation and a proliferative phase during the remainder of the cycle. Microscopically, the prolifera-

9. Moszkowicz, L.: Arch. klin. Chir. **142**:374, 1926.
10. Dawson, E. K.: Edinburgh M. J. **41**:653, 1934.
11. Taylor, H. C., Jr.: Surg., Gynec. & Obst. **62**:129, 1936.
12. Taylor, H. C., Jr., Surg., Gynec. & Obst. **62**:562, 1936.
13. Ingleby, H.: Arch. Path. **14**:21, 1932.
14. Ingleby, H.: Arch. Path. **33**:573, 1942.

tive stage is characterized by edematous, pale-staining stroma with young fibroblasts and lymphocytes, as well as proliferation of the duct system and epithelium. The regressive stage does not include loss of lobular structure, although there are involuntionary changes with formation of hyalinized stroma. Geschickter found proliferation of periductal stroma similar to that seen in the normal adolescent female breast after the administration of estrogen to mature women.

Leo Loeb [15] studied the stroma of the breast in guinea pigs with particular interest, especially with regard to the relationship between breast epithelium and stroma. He suggested that a close physiological similarity between terminal ducts and alveoli is indicated by the stroma, which is similar for the two. A relationship between the activity of the glandular elements and the surrounding connective tissue was found. Where the epithelium proliferates, there is corresponding activity in the intralobular stroma, although the more distant interlobular stroma remains fibrous. On the other hand, the large ducts remain in a resting state, and the surrounding stroma is fibrous.

In an early study Kuru [16] found the breast stroma to be metachromatic with polychrome methylene blue under certain conditions. Metachromasia was found in periductal stroma of newborn and infant breasts and in the subepithelial zone of the fibroadenomas; the stroma of normal and mature breasts, lactating breasts, and adenomas was not metachromatic. Kuru believed that metachromasia of the stroma indicated mucinous degeneration. In subsequent reports this staining reaction was said to be nonspecific, unreliable, and of little significance,[8] or it was used as evidence for the specialized nature of this tissue.[9]

More recently, several reports [17] have described metachromasia of lobular stroma with use of the toluidine blue stain. It is partially removed after incubation with testicular hyaluronidase and is presumably due to the presence of mucopolysaccharides. Mancini and co-workers [18] studied the interstitial substance and the changes in fibers of fibroadenomas. Metachromatic interstitial material was most abundant when fibers were scarce, as Kuru had stated. Fibroadenomas showing dense hyaline connective tissue showed almost no ground substance. Metachromasia was inhibited by incubation with hyaluronidase. Mancini and co-workers suggest that interstitial ground substance is derived from disintegrating collagen fibers.

MATERIAL AND METHODS

All material studied consisted of surgical material from the files of the Barnard Free Skin and Cancer Hospital. Hematoxylin and eosin sections were first scanned without specific knowledge of the diagnosis or other clinical information; slides were selected to illustrate an arbitrary series of changes which had been observed during the routine study of these specimens. These changes included those which have been described as the stromal changes occurring in the menstrual cycle; further selection was made to include other stromal changes commonly observed in surgical material. Fibroadenomas were included because of the prominent stromal

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16. Kuru, H.: Deutsch. Zeitschr. Chir. **98**:414, 1909.

17. Sylven, B.: Arch. path. Anat. **303**:280, 1938. Wislocki, G. B.; Bunting, H., and Dempsey, E. W.: Am. J. Anat. **81**:1, 1947. Dempsey, E. W.; Bunting, H., and Wislocki, G. B.: ibid. **81**:309, 1947. Bunting, H.: Ann. New York Acad. Sc. **52**:977, 1950.

18. Mancini, R. E.; Bur, G.; Brandes, D., and Garberi, J. C.: Rev. Soc. argent. biol. **27**:243, 1951.

component of these growths. Neutral formalin in 10% solution was the fixative used in all instances. Since the diagnoses and clinical data were diregarded, with the exception of fibroadenomas, several blocks were occasionally selected from the same patient to illustrate different features. Thus, 27 blocks were studied from 21 different patients. Eleven fibroadenomas were studied.

Staining procedures and histochemical methods were selected to give a rather complete picture of the connective tissue elements. They include the Verhoeff-Van Gieson stain, the phosphotungstic acid hematoxylin stain, and Wilder's staining method for reticulum. Periodic acid-Schiff stains were made on selected specimens. No preparations were made with the periodic acid-Schiff procedure after treatment with hyaluronidase. Sections from all patients were stained with toluidine blue[19] after incubation for 18 hours at 36 C. with active and inactive testicular hyaluronidase (Wydase, which was inactivated by boiling for 10 minutes); in selected instances tissues were similarly treated after incubation with streptococcal hyaluronidase.[20]

Preliminary testing with these enzymes indicated that between 500 and 1,000 TRU (turbidity-reducing units) in saline at pH 5.0 was necessary to remove metachromasia in some instances. Accordingly, 750 TRU in saline at pH 5.0 of the two enzymes was used.

The material used has several definite limitations which in part determined the approach used. These limitations should be constantly kept in mind in interpretation of results. In the sense that all the material was obtained from women who had some variety of breast complaint, all of the material is abnormal. Menstrual data of sufficient detail are so rarely available in the clinical records that no attempt was made to correlate changes seen with menstrual phases. It is possible that results may differ in material obtained from healthy, normally menstruating women. Also, fixation varies considerably in this type of material; in some cases fixation is more rapid than in others. Moreover, the fixative used is not optimal for some of the staining procedures.

RESULTS

It is apparent that the "normal" amount and type of stroma for a lobule must be defined in terms of several factors if the above reports in the literature are accepted. However, if lobules are selected in which the amount of stroma is in average proportion to the epithelial elements, one may find cellular, collagenous, or fibrillary stroma. Further, the stroma may be increased beyond the rather arbitrarily defined normal amount. In some instances a greater increase in stroma is associated with distortion of the structure within the lobules. The latter changes cannot be separated from small fibroadenomas. Specific examples were selected upon the basis of this general outline; the variations seen and the results of the special procedures will be described.

Lobules in which the stroma is designated as normal in amount often have a cellular appearance and are sharply set off from surrounding connective tissue (Fig. 1*A*). The lobular stroma has a loose texture with prominent interstitial spaces. The cells have the features of rather small fibroblasts. The nuclei are either somewhat elongated or oval, although they are not usually hyperchromatic. In stroma of this type, extracellular fibers are not recognizable, but short cellular processes or fibrils are easily seen. These processes show a variable amount of orientation which is generally slight, however. The reticulum stain (Fig. 1*B*)

19. Methods for determining metachromasia vary considerably. After testing varying concentrations and periods of staining, this routine was used: a 0.5% solution of toluidine blue in water at pH 4.5 to 5.0 for four to five minutes, followed by one rapid wash in tap water, two changes in 95% alcohol for five seconds each, one change in absolute alcohol for five seconds, alcohol-xylene and xylene changes for one minute, and mounting in balsam.

20. This enzyme (Lot A-461) was supplied by Dr. Joseph Seifter, Wyeth Institute of Applied Biochemistry, Philadelphia.

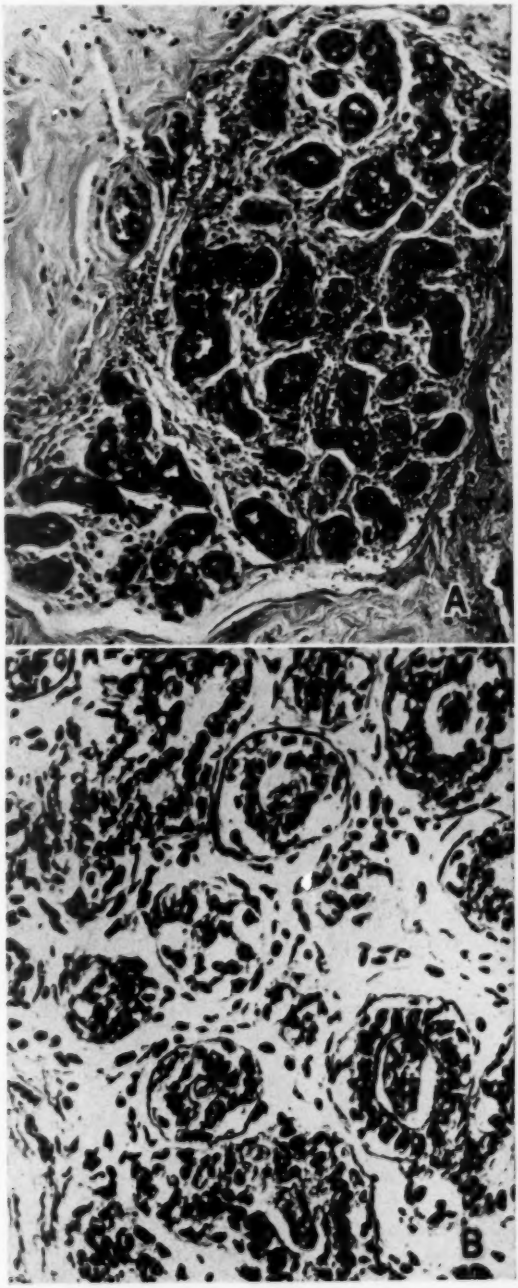

Fig. 1.—*A*, Loose cellular lobular stroma. Hematoxylin and eosin; × 150. *B*, reticulum stain of Figure 1*A*. Note staining of basement membranes and lack of reticulum fibers; × 320.

sharply outlines the basement membranes of the tubules; however, it stains fewer of the cytoplasmic fibrils than does the hematoxylin and eosin preparation. Likewise, phosphotungstic acid hematoxylin and Van Gieson stains demonstrate these processes poorly. Occasional plasma cells, lymphocytes, and mononuclear macrophages contribute to the cellularity of the lobules; the average number of these cells is small.

In contrast, lobules of similar size are easily found in which the stroma is largely composed of dense collagen bundles (Fig. 2*A*). Actually, the amount of collagen fibers is quite variable and may range from scattered strands in a looser and more cellular background to lobules in which the stroma is entirely hyalinized and acellular. Also, fibrillary structure may be more or less distinct. Although no striking orientation is seen, tubules are often surrounded by loops of collagenous material. In some lobules collagen bands and cellular stroma are intermingled. Bands of collagen at the lobular margins are often continuous with the interlobular stroma; in contrast, lobules with cellular stroma are sharply separated. The collagen in these lobules shows the characteristic staining reactions with phosphotungstic acid hematoxylin, Van Gieson's stain, and Wilder's reticulum stain (Fig. 2*B*). Most lobules in which collagen is present show more connective tissue cells than one might expect in perilobular tissue of this type. These cells often have fairly plump oval nuclei, rather than the thin elongated nucleus of the mature fibroblast.

A third type of lobule exhibits stroma which is fibrillary and intermediate in structure between the types just described, although the amount of stroma need not differ (Fig. 3*A*). Fibrils and fibers are a more prominent feature of these lobules. They range from thin delicate fibrils, which are cytoplasmic processes in some instances, to fibers which are of the order of reticulum fibers. However, a constant feature of these larger fibers is the failure to stain black with the reticulum stain (Fig. 3*B*); rather, they take the same gray tones as the interlobular collagen. With the Van Gieson stain a reddish hue is sometimes retained, although they may also take a very pale gray color. With phosphotungstic acid hematoxylin they stain pale gray-brown. There is usually a rather definite parallel arrangement of these fibers which tend to form loops around tubules. As noted, mixtures of this type of stroma with varying amounts of collagen may be seen. The cellularity of these lobules is not constant, although it tends to be greater than in the other two groups. Spindle-shaped cells are most numerous; the plumper cells described earlier may also be found.

Metachromasia with the toluidine blue stain follows no sharply drawn patterns. Contrary to anticipated findings, metachromasia is generally not present in lobules showing loosely cellular or fibrillary stroma. On the contrary, in our material it is more constantly present in the stroma which is predominantly collagenous. Furthermore, there is no consistent relationship between metachromasia and basophilia as determined with hematoxylin and eosin stains. In one instance, lobular stroma was almost acellular and consisted of broad interlacing bands of dense collagen; in spaces between these bands metachromatic material was stained. There is no detectable relationship between metachromasia and the presence of cells in the lobular stroma. Metachromasia of the lobular stroma is always removed by incubation with testicular hyaluronidase but not affected by streptococcal hyaluronidase. Collagenous areas in the walls of cystic ducts are also metachromatic in some instances; here

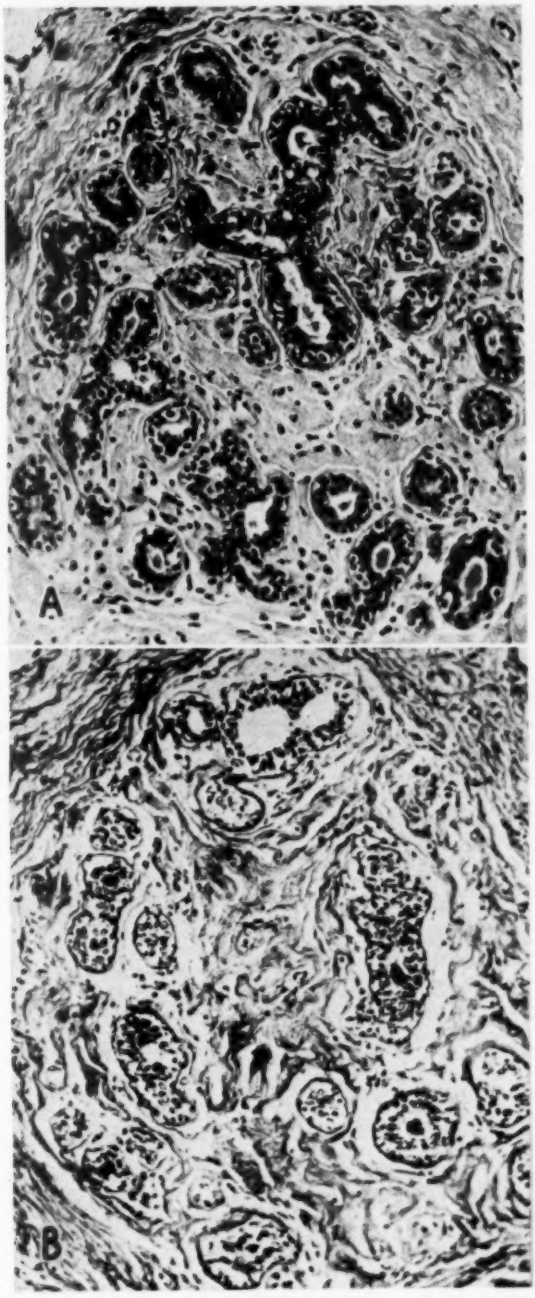

Fig. 2.—*A*, collagenous lobular stroma. Hematoxylin and eosin; × 150. *B*, reticulum stain of Figure *2B*; × 150.

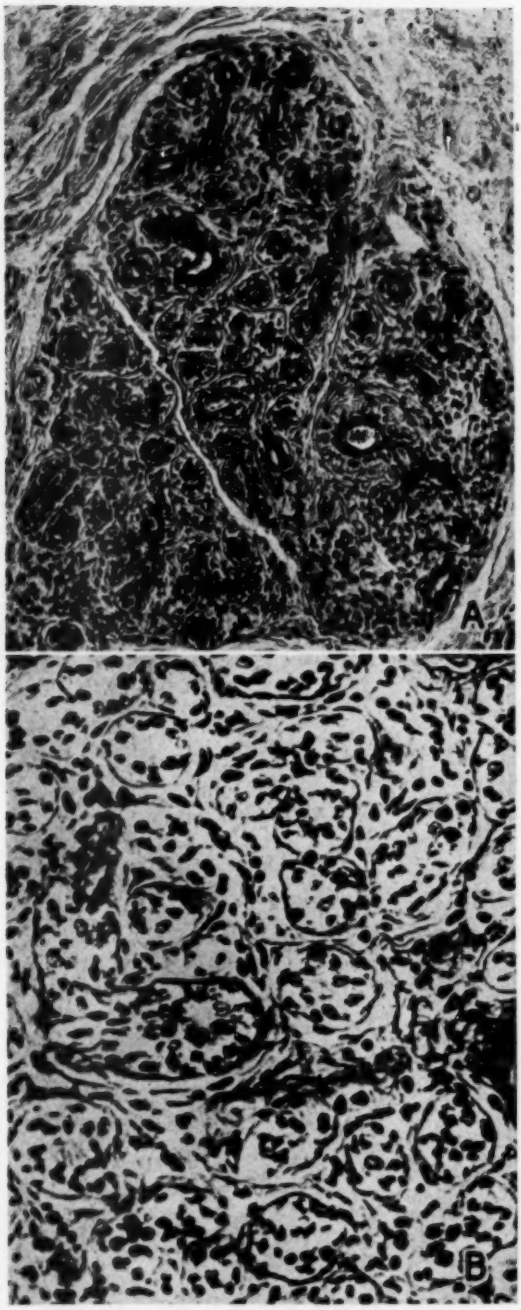

Fig. 3.—*A*, fibrillary lobular stroma. Hematoxylin and eosin; × 150. *B*, reticulum of Figure 1*A*. Compare sharply outlined basement membranes with lack of staining of interstitial fibers; × 320.

the material is not always removed by enzymatic action. Material within duct lumens is generally deeply metachromatic. It is consistently not affected by the enzymes. Mast cells were identified by metachromatic granules, and metachromasia was regularly retained after incubation with hyaluronidase.

With the periodic acid-Schiff procedure, none of the lobular stromal structures stained red, with the exception of the basement membranes which were brilliantly outlined. Duct contents were also positive with this procedure.

These types of intralobular stroma are by no means pure forms in the material we have examined, as has been stated; transitions and mixtures are easily found. It will be noted that the cellular (Fig. 1*A*) and intermediate fibrillary (Fig. 3*A*) types correspond to the descriptions of the lobular stroma during premenstrual and menstrual phases, as well as to the stroma in mazoplasia. The collagenous stroma (Fig. 2*A*) is similar to that described as occurring in the interval phase in the menstrual cycle.

In lobules which show more stroma than those just described and in which the amount seems out of proportion to the epithelial elements, the same types of connective tissue may be found. Thus the stroma may be loose and cellular (Fig. 4*A*), fibrillary (Fig. 4*C*), or collagenous (Fig. 4*B*). The same mixtures are also found. The results of the special stains and procedures differ in no way from those in which the stroma is considered to be normal in amount. These lobules may be considered to show hyperplasia of stroma. We did not find satisfactory examples of atrophy of stroma unless there was also an evident decrease in tubules.

In addition to lobules which show stromal hyperplasia, one may find lobules in which hyperplasia and tubular distortion suggest the structure of fibroadenomas (Fig. 5). These lobules are usually larger than those designated as hyperplasia. Rather long segments of tubules with compressed or slit-like lumens are seen. For descriptive purposes these structures have been designated as microfibroadenomas. One may find transitions between lobules which show distortion of only a few tubules and others which cannot be distinguished from fibroadenomas. The stroma may be fibrillary or more densely collagenous. At this point we have not found a microfibroadenoma in which the stroma is loose and cellular; however, on the basis of the types of stroma which may be found in a fibroadenoma, we feel certain that such examples exist. The results of special stains and techniques on microfibroadenomas do not differ from those obtained in the groups which have been described.

The examples of fibroadenomas which we have examined exhibit stroma which is quite similar to the varieties of connective tissue which are seen within lobules; the only differences are quantitative. At one extreme, the stroma is poorly cellular (Fig. 6*A*); long thin fibers are related to spindle cells which are oriented in a rather definite pattern. These fibers are commonly perpendicular to the long axis of the epithelium and tend to converge into loose fibrillary bands in central areas. In this type of stroma, there is abundant interstitial material between fibers which is unstained or slightly basophilic with hematoxylin and eosin. Occasionally rather large pools of this material are seen in which cells and fibers are scanty. As is the case in lobules, these delicate fibers are more distinctly stained with hematoxylin and eosin than with the phosphotungstic acid hematoxylin, Van Gieson, or reticulum stains (Fig. 6*B*). With the Van Gieson stain they occasionally take a reddish hue. With the reticulum stain they stain the same gray hue as the adjacent collagen.

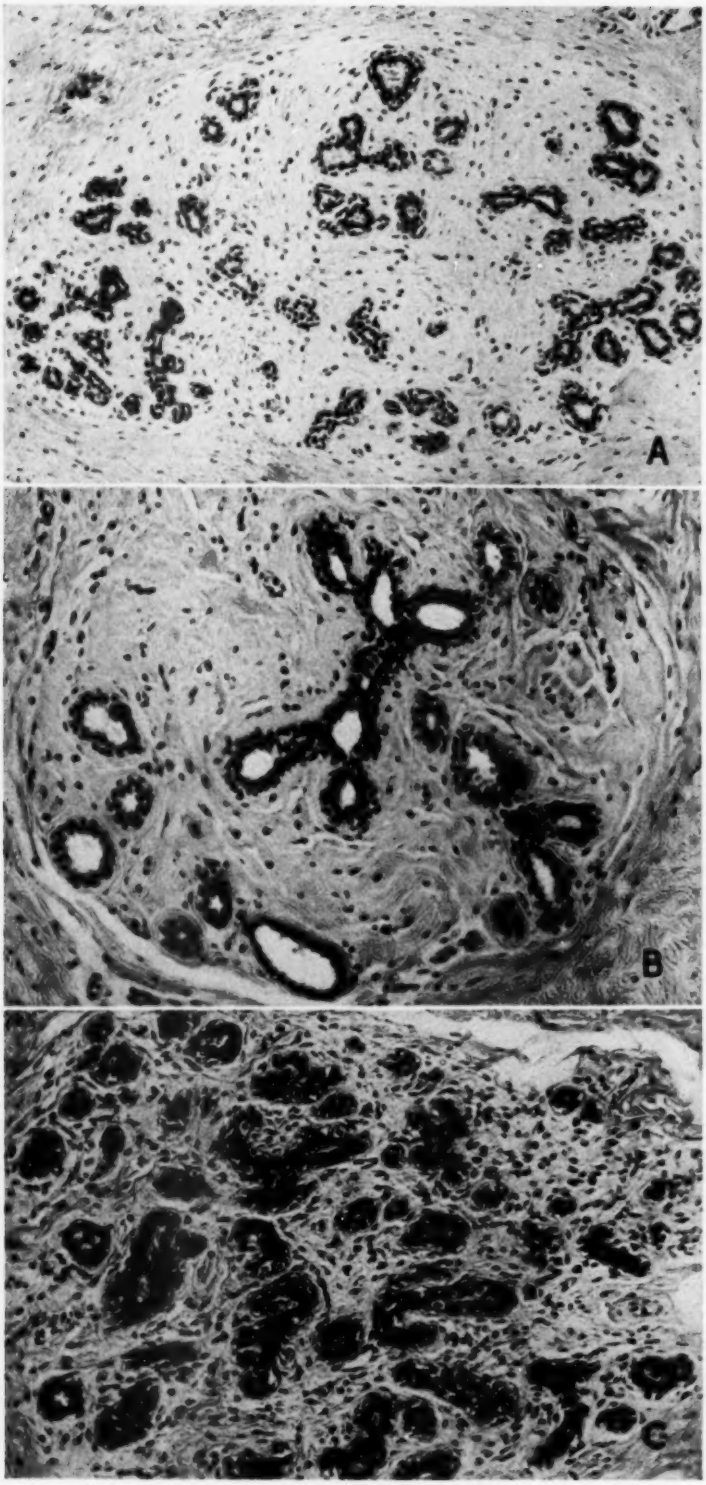

Fig. 4.—*A*, hyperplasia of loose lobular stroma. Hematoxylin and eosin; × 105. *B*, hyperplasia of collagenous lobular stroma. Hematoxylin and eosin; × 105. *C*, hyperplasia of fibrillary lobular stroma. Hematoxylin and eosin; × 105.

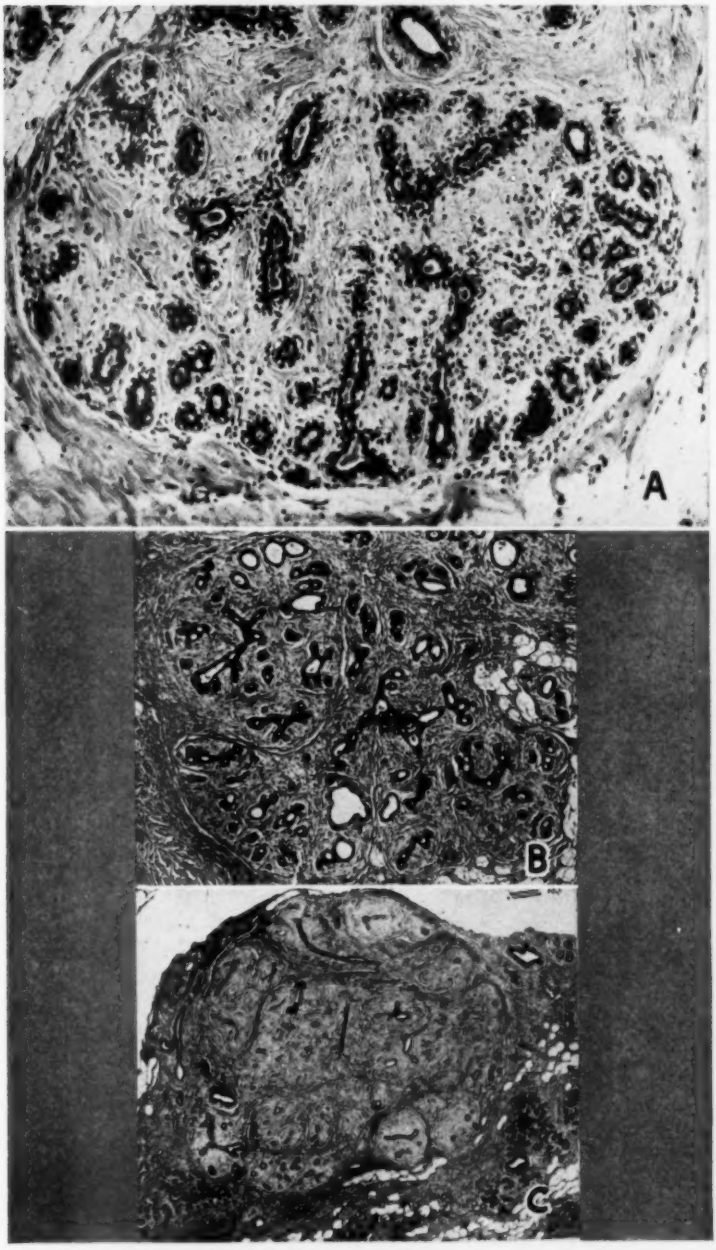

Fig. 5.—*A*, hyperplasia of lobular stroma with slight tubular distortion. Hematoxylin and eosin; × 105. *B*, hyperplasia of lobular stroma and distortion of tubules; resembles microscopic fibroadenoma.. Hematoxylin and eosin; × 30. *C*, more marked stromal hyperplasia; microscopic fibroadenoma. Hematoxylin and eosin; X 15.

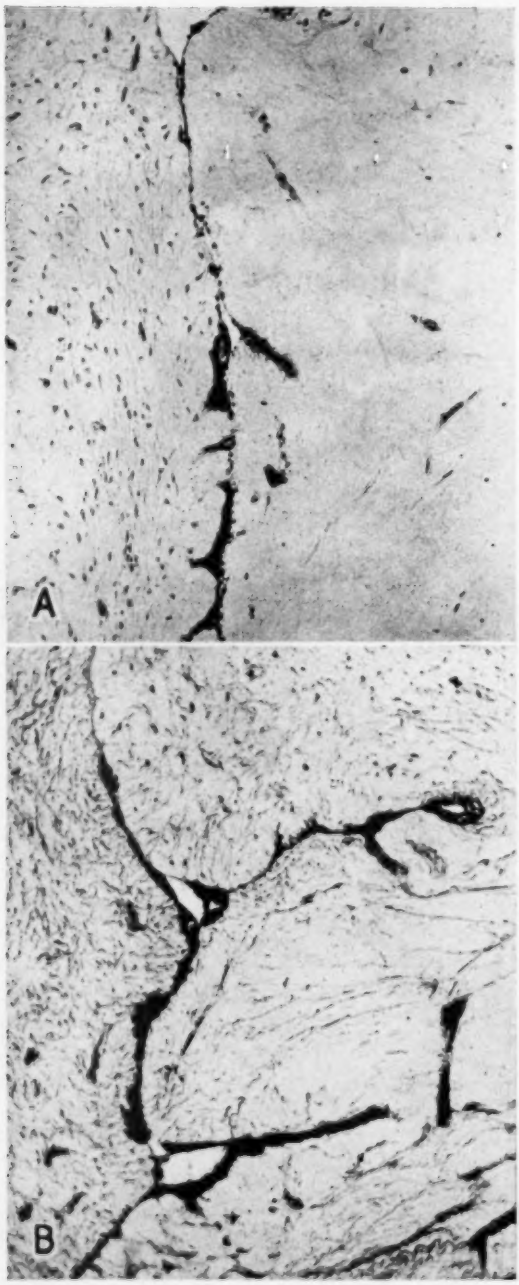

Fig. 6.—*A*, fibroadenoma; loose stroma with abundant interstitial material; stroma to right of compressed tubule almost acellular. Hematoxylin and eosin; × 150. *B*, reticulum stain of Figure 6*A* (level differs slightly). Note lack of reticulum fibers; × 150.

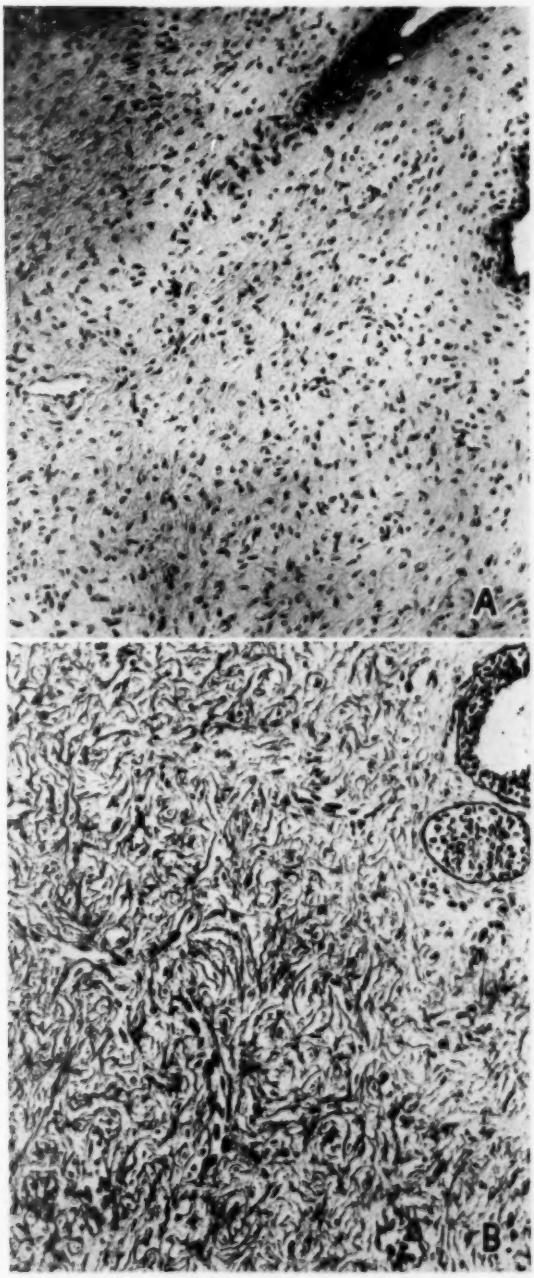

Fig. 7.—*A*, fibroadenoma; stroma cellular; abundant interstitial material. Hematoxylin and eosin; × 150. *B*, reticulum stain of Figure 7*A*. Compare black basement membrane in upper right with dark gray stromal fibers; × 150.

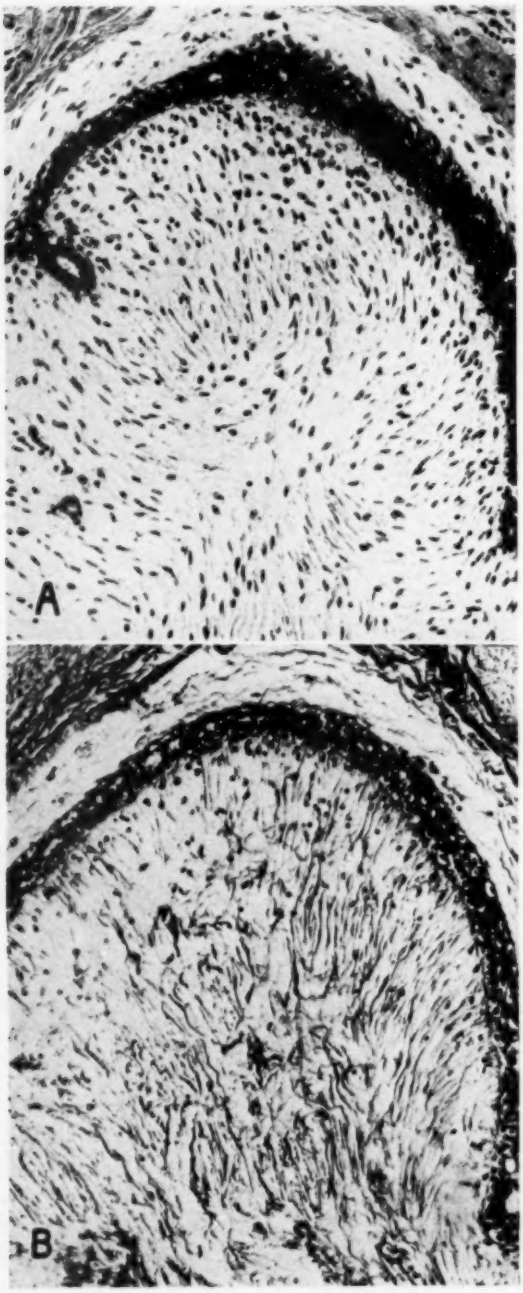

Fig. 8.—*A*, fibroadenoma; fibrillary stroma. Hematoxylin and eosin; × 150. *B*, reticulum stain of Figure 8*A*; × 150.

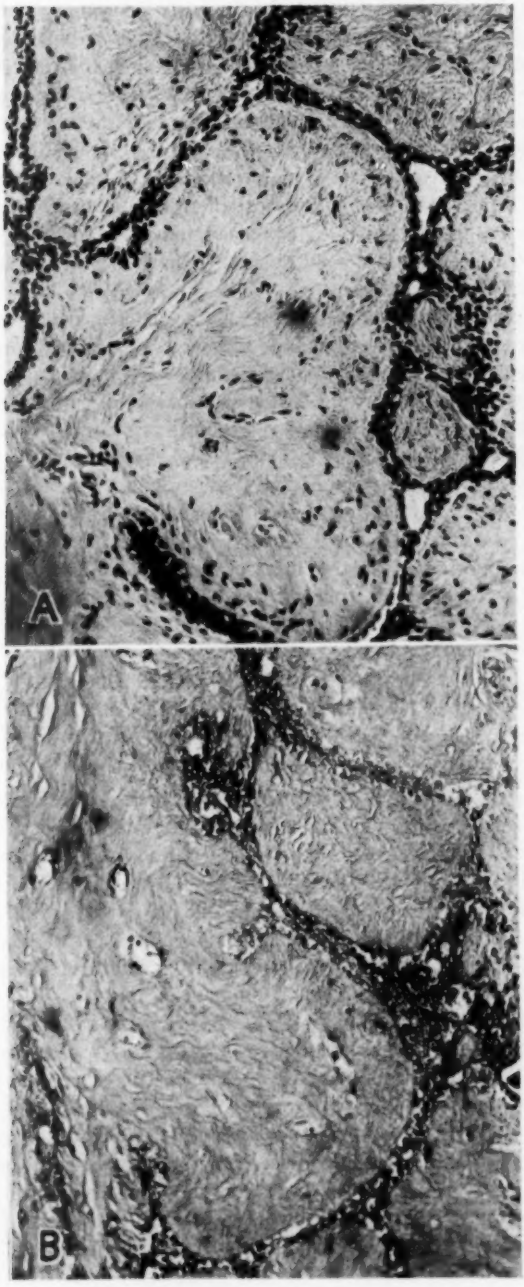

Fig. 9.—*A*, fibroadenoma; collagenous stroma. Hematoxylin and eosin; × 150. *B*, reticulum stain of Figure 9*A*; × 150.

The appearance of the cells differs little from that of cells in comparable lobular stroma; the cells are frequently thinner, and the nuclei stain more deeply. In one specimen the stroma was very cellular and less distinctly fibrillary (Fig. 7*A*). In this instance the reticulum stain (Fig. 7*B*) revealed an unorderly network of fibers which again had the gray hue of collagen. One of the commoner varieties of stroma corresponds to the type designated as fibrillary in lobules (Fig. 8*A*). The fibrillary aspect of this tissue is the most prominent distinguishing feature. These fibers again stain poorly and are not argyrophilic (Fig. 8*B*). Finally, fibroadenomas containing densely collagenous or hyaline stroma are also extreme examples (Fig. 9*A*). In these instances, both staining reactions and structure identify this material as collagen (Fig. 9*B*).

With few exceptions, interstitial material in the stroma of fibroadenomas is heavily metachromatic after toluidine blue staining. This is particularly true in those sections which show abundant pale or slightly basophilic material between the widely separated fibers. The degree of metachromasia varies from area to area and cannot always be predicted on the basis of structure or staining reaction. In general, loose stroma adjacent to epithelium is more heavily metachromatic than the denser peripheral tissue. The latter areas are frequently not metachromatic. Several fibroadenomas which contained densely collagenous or hyalinized stroma revealed no metachromatic material. Testicular hyaluronidase regularly reduces the amount of metachromasia; in most instances metachromasia is completely removed. Where metachromasia is not removed, small foci of pink remain in areas which were previously heavily metachromatic. Streptococcal hyaluronidase has no effect on metachromasia.

Areas which are metachromatic with toluidine blue are regularly Schiff-negative. Again, the periodic acid-Schiff stain outlined the basement membranes of tubules in bright red hues.

COMMENT

From the anatomical studies recorded in the literature, it could be postulated that intralobular and periductal stromata follow the general morphological sequence of connective tissue maturation. Thus, during periods of proliferation or activity of the breast, the stroma is cellular with interstitial material. Fibers then become more numerous; collagen is formed during inactive periods while the fibroblasts become small. Periods of proliferation, which have been described, include embryonic development, birth, puberty, the premenstrual and menstrual phases of the cycle, some areas of lactating breasts, and gynecomastia. Fibroadenomas are examples in which proliferation is excessive to the point of tumor formation. The stroma in these structures is classically loose and cellular. The inactive or involutionary periods include the interval phase of the menstrual cycle and postmenopausal atrophy. During the inactive periods the collagen within lobules is structurally indistinguishable from the supporting nonreactive interlobular stroma.

A healing wound is perhaps the most easily studied example of maturation of connective tissue; many studies[21] have indicated that after initial edema and appearance of ground substance, circulating leucocytes appear. Proliferation of fibroblasts follows quickly; reticulum fibers then appear and are followed by thick,

21. (*a*) Sylven, B.: Acta. chir. scandinav. (Supp. 66) **8**:1-151, 1941. (*b*) Perez-Tamayo, R., and Ibnen, M.: Am. J. Path. **29**:233, 1953.

mature collagen. Metachromasia of the ground substance with toluidine blue appears during the initial phase, when interstitial material is abundant. With proliferation of fibroblasts metachromasia disappears, and no metachromatic material is seen when mature collagen has been formed. The material associated with metachromasia has been identified as mucopolysaccharides, chiefly hyaluronic acid and chondroitin sulfate. Identification is based on loss of metachromasia following incubation with streptococcal or testicular hyaluronidase; these enzymes are assumed to act specifically on hyaluronic acid and chondroitin sulfate as a substrates.

It seemed a reasonable assumption that metachromasia of breast stroma would be associated with the loosely cellular or fibrillary types which are described as the types of stroma in periods of proliferation. However, the slides which we selected to correspond to these stromal types (Figs. 1*A* and 3*A*) generally did not show stromal metachromasia. On the contrary, metachromasia was more commonly seen in lobules with collagenous stroma (Fig. 2*A*). Fibroadenomas, on the other hand, regularly exhibited metachromasia if the stroma was loose and contained abundant interstitial material; this stroma is not necessarily cellular, however, and the histological appearance is not always conspicuously that of proliferation. It would be of considerable interest to determine if so-called microfibroadenomas with loose cellular stroma show stromal metachromasia; lobules with hyperplasia of loose cellular stroma do not show metachromasia in our experience.

The enzyme studies established the nature of the major portion of the material which stained metachromatically. Since it is possible that other mucopolysaccharides might be correlated with the interstitial metachromasia, selected slides were stained with the periodic acid-Schiff procedure, which is used by some to demonstrate mucopolysaccharides and glycoproteins. The lobular and periductal stroma was consistently Schiff-negative in our material.

Thus, comparison of lobular or periductal stroma with growing connective tissue indicates an important difference, i. e., in the relationship of metachromatic material to the other connective tissue elements. However, if the cyclic stromal changes in the breast described in the literature are accepted, there is an even more fundamental difference. Collagen fiber formation in a healing wound represents the final inactive stage. However, collagenization in the lobular stroma of the breast is replaced by cellular connective tissue during the subsequent menstrual cycle. It is generally held that once collagen is formed it remains indefinitely; it is never reabsorbed unless preceded by necrosis (a possible exception [22] has been reported). In fact, available information indicates that collagen is metabolically almost inert.[23] Practically no attempt has been made by authors describing stromal changes in the breast to explain how this collagen is removed, and no morphological evidence has been presented. There is little evidence to support the explanation by Dieckman that collagen is masked and unmasked as fluid is transferred from interstitial space to collagen. We have recognized no histological evidence of removal of collagen in our material.

22. Cameron, G. R., and Karunaratne, W. A. E.: J. Path. & Bact. **42**:1, 1936. Steinberg, B., and Martin, R. A.: Arch. Path. **41**:1, 1946. Morrione, T. G.: J. Exper. Med. **85**:217, 1947. Morrione, T. G.: Am. J. Path. **25**:273, 1949.

23. Neuberger, A.; Perrone, J. C., and Slack, H. G. B.: Biochem. J. **49**:199, 1951.

At this point it is again necessary to call attention to the limitations of our material. Water-soluble mucopolysaccharides may be lost in formalin-fixed material. Diseases encountered in surgical specimens may have an unrecognized influence. In other words, it is possible that the relationship of metachromasia to other stromal elements may differ in optimal material; this possibility is not likely in our opinion because our heterogeneous material showed certain trends. Aside from the problem of metachromasia, the fate of collagen in lobular and periductal stroma remains to be explained. It is apparent that a careful study of breasts from normal healthy women is needed to establish the stromal changes associated with the menstrual cycle.

From another point of view, breast stroma may be compared with those tissues which react markedly to hormonal stimulation. These tissue areas have been described in various animal species and are usually, but not always, related to reproductive organs. A number of features are common to these areas of connective tissue. The available information is presented in the Table; our results are included for comparison.

The skin of the rhino mice treated with folliculoids reacts in a similar manner.[24] It has not been included because the experimental situation is rather artificial and because the reaction does not occur in any special area of connective tissue. Changes in the skin in myxedema,[25] which have been more thoroughly studied, are apparently secondary to the absence of hormonal stimulation. The role of thyrotropin in the production of pretibial myxedema has recently been contested.[26]

Cellular changes described are proliferation, enlargement, and hyperchromatism of fibroblasts. An exception is the fibroblasts of the orbital tissue[27] of the thyroidectomized guinea pig; these fibroblasts showed no changes. These cellular changes are often quite striking. In the material we have reviewed cellular changes occurred, but they were not impressive. They consisted of variations in the number of cells in lobules with stroma of varying types, as well as variations in the size and number of cells in fibroadenomas.

A distinguishing feature of these areas of connective tissue is the presence of abundant interstitial metachromatic material. Metachromasia is almost completely inhibited by testicular hyaluronidase, with the exception of the endometrium; in contrast, streptococcal hyaluronidase is only partially successful in removal of metachromatic material in instances in which it has been used. The results of enzymatic digestion indicate that the metachromatic material contains hyaluronic acid and chondroitin sulfate. Hyaluronic acid has been isolated from the sex skin and the cock's comb; the orbital contents of the guinea pig have a high hexosamine content. The types of stroma in which metachromatic material was found in our sections have been described. In our material, no relationship was observed between the

24. Seyle, H.: Arch. Dermat. & Syph. **50**:261, 1944.

25. Mancini, R. E.; Gadberi, J. C., and de la Balze, F. A.: Rev. Soc. argent. biol. **27**:285, 1951. Pearce, R. H., and Watson, E. M.: Canad. J. Res., Sect. E **27**:43, 1949. Watson, E. M., and Pearce, R. H.: Am. J. Clin. Path. **19**:442, 1949. Brewer, D. B.: J. Path. & Bact. **63**:503, 1951.

26. Gabrilove, J. L.: Ludwig, A. W., and Soffer, L. J.: Effect of Thyroid Hormone and Thyrotropin on the Ground Substance and Connective Tissue, read before the Thirty-Fourth Meeting of the Endocrine Society, Chicago, June, 1952.

27. Klemperer [2] stated that the retroperitoneal tissue reacted in the same way.

Summary of Available Information on Connective Tissue Areas Responding Readily to Hormones

Organ	Animal	Source of Hormonal Stimulus	Cellular Changes	Fiber Changes*	Interstitial Metachromasia	Testicular Hyaluronidase	Streptococcal Hyaluronidase	Periodic Acid-Schiff-Positive Material	Chemical Analysis
Sex skin†	Monkey	Estrogen	Enlargement, hyperchromatism, and proliferation of fibroblasts	Thinning or dissociation of collagen fibers	Present	Removed	Partially removed	?	Hyaluronic acid, water
Comb‡	Cock	Estrogen	Enlargement, hyperchromatism, and proliferation of fibroblasts	Dissociation of collagen fibers	Present	Removed	Partially removed	Absent	Hyaluronic acid
Symphysis pubis§	Guinea pig	Pregnancy, relaxin	Proliferation of fibroblasts	Thinning or "dissolution" of collagen fibers and cartilage	?	?	?	Present	?
Orbital and retroperitoneal tissue‖	Guinea pig	Thyroidectomy and TSH (thyroid-stimulating hormone	None	Dissociation of collagen fibers	Present	Removed	Partially removed	Present	High hexosamine, water
Uterus, cervix, and vagina¶	Rat	Estrogen	Change in shape from round to spindle-shaped fibroblasts	Change from reticulum to collagen; deposit of fibrohyaline material	?	?	?	?	?
Endometrium#	Rat, human	Menstrual cycle	Proliferation of fibroblasts	Proliferation of reticulum fibers	Present	Not removed	?	?	?
Endometrium**	Rat	Steroids, trauma	Enlargement and proliferation of fibroblasts	Dissociation and thinning of collagen fibers	?	?	?	?	?
Ovary††	Rabbit, guinea pig	Pregnancy, gonadotropins	Complex cellular rearrangements during cycle	Change from collagen to reticulum	Present	?	?	Present	?
Fallopian tube, mesosalpinx‡‡	Rat	Steroids	Stellate cells	Not described	?	?	?	?	?
Ejaculatory ducts§§	Mice	Estrogens	Stellate cells	?	?	?	?	?	?
Breast‖‖	Guinea pig	Menstrual cycle	Proliferation of fibroblasts	Change from collagen to thinner fibers	Present	Removed	?	?	?
Breast	Human	Menstrual cycle	Variation in size and number	Presence of collagen or thinner fibers	Variable	Removed	Not removed	Absent	?
Breast (fibroadenoma)	Human	?	Variable, occasionally large	Thin fibers to hyaline collagen	Present	Almost completely removed	Not removed	Absent	?

* Our interpretation of changes seen.

† Collings, M. R.: Anat. Rec. **33**:271, 1926. Allen, E.; J. Morphol. **46**:479, 1928. Bachman, C.; Collip, J. B., and Selye, H.: Proc. Roy. Soc. Med. **117**:16, 1935. Aykroyd, O. E., and Zuckerman, S.: J. Physiol. **94**:13, 1938. Duran-Reynals, F.; Bunting, H., and van Wagenen, G.: Ann. New York Acad. Sc. **52**:1006, 1950.

‡ Champy, C., and Kritch, N.: Compt. rend. Soc. biol. **92**:683, 1925; Compt. rend. Acad. Sc. **180**:957, 1925. Hardesty, M.: Am. J. Anat. **47**:277, 1931. Szirmai, J. A.: Anat. Rec. **105**: 337, 1949. Boas, N. F.: J. Biol. Chem. **181**:573, 1949. Boas, N. F., and Ludwig, A. W.: Endocrinology **46**:289, 1950; ibid. **46**:291, 1950. Schiller, S.; Benditt, E. P., and Dorfman, A.: ibid. **50**:504, 1952.

§ Ruth, E. B.: Anat. Rec. **67**:69, 1936; ibid. **67**:409, 1936. Talmage, R. V.: ibid. **99**:91, 1947. Perl, E., and Catchpole, H. R.: Arch. Path. **50**:233, 1950.

‖ Ludwig, A. W.; Boas, N. F., and Soffer, L. J.: Proc. Soc. Exper. Biol. & Med. **73**:137, 1950.

¶ Loeb, L.; Suntzeff, V., and Burns, E. L.: Science **88**:432, 1938; Am. J. Cancer **35**:159, 1939. Suntzeff, V.; Babcock, R. S., and Loeb, L.: ibid. **38**:217, 1940. Burack, E.; Wolfe, J. M., and Wright, A. W.: Endocrinology **30**:335, 1942.

Bensley, S. H.: Anat. Rec. **60**:93, 1934. Holmgren, H.: Ztschr. mikr-anat. Forsch. **47**:489, 1940. McKay, D. G.: Am. J. Obst. & Gynec. **59**:875, 1950.

** Selye, H.; Harlow, C., and McKeown, T.: Proc. Soc. Exper. Biol. & Med. **32**:1253, 1935. Selye, H., and Friedman, S.: Am. J. Cancer **38**:558, 1940.

†† Duke, K. L.: Anat. Rec. **98**:597, 1948. Harter, R.: ibid. **102**:349, 1948. Catchpole, H. R.; Gersh, I., and Pan, S. C.: J. Endocrinol. **6**:277, 1950.

‡‡ Selye, H.: Anat. Rec. **76**:145, 1940.

§§ Burrows, H.: J. Path. & Bact. **41**:423, 1935.

‖‖ Loeb, L., and Simpson, R. M.: Science **88**:433, 1938. Loeb, L., and Hesselberg, C.: J. Exper. Med. **25**:285, 1917.

presence of metachromasia and cellular proliferation. This supports the opinion expressed elsewhere [28] that the deposition of metachromatic material is apparently independent of cells.

The presence of Schiff-positive material is less constant than that of metachromatic substnces, and it bears no apparent relationship to the latter. It may be significant that Schiff-positive material is not removed by hyaluronidase, possibly indicating that this stain reveals chemical groups which are different from those revealed by the metachromatic stains.

Changes in connective tissue fibers have been given insufficient attention in these reports. Staining quality, argyrophilia, and the changes in fibers when metachromatic material appears are examples of details which are lacking. True proliferation apparently occurs only in the endometrium. In our results the most significant features with regard to fibers is the inability to stain reticulum fibers in lobular stroma, as well as in fibroadenomas. In general, the fibers were somewhat more delicate than reticulum fibers in areolar tissue. However, fibers showing no structural differences from reticulum fibers also failed to stain black with silver. We can offer no satisfactory explanation.

The hypothesis has been stated [29] that changes in the connective tissue of glandular organs are secondary to changes in the epithelium. This hypothesis is obviously not tenable for those areas without glandular structures (sex skin, cock's comb, symphysis pubis, orbital and retroperitoneal tissue, vagina, mesosalpinx) where the changes are more probably secondary to direct hormonal action. However, the suggested relationship between connective tissue and epithelium in such organs as the endometrium, breast, uterus, Fallopian tube, and ejaculatory ducts cannot be excluded.

In summary, this heterogeneous group of areas of connective tissue is specialized in the sense that it responds in an exaggerated way to hormonal stimulation. The morphology of the reaction is characterized by the presence of abundant metachromatic material (hyaluronic acid, chondroitin sulfate, and possibly other substances), cellular hyperplasia and hypertrophy, and fibrillary changes. Furthermore, these changes seem to follow a definite sequence: cellular stimulation and deposition of metachromatic material occur simultaneously, and the changes in the character of the fibers appear and progress as they are separated by the metachromatic substance. Since, with the exception of the endometrium, there is no formation of new connective tissue, it is difficult to accept the suggested hypothesis that metachromatic material participates in the synthesis of fibers or that it acts as an interfibrillary cement [30] in these tissues.

Several features indicate that the intralobular and periductal breast stromata are tissues belonging to this general group. Although the hormonal stimuli may not be precisely defined, it is apparent that important stromal changes take place in these areas during major hormonal shifts such as birth, puberty, and menopause. It is likely that menstrual changes of some type also exist. Further, during these periods of hormonal response this tissue is structurally quite different from the

28. Perez-Tamayo, and Ibnen.[21b] Altschuler, C. H., and Angevine, D. M.: Am. J. Path. **25**:1061, 1949. Scott, V., and Dammin, G. J.: Am. J. Syph. **34**:501, 1950.

29. Loeb, L., and Simpson, R. M.: Science **88**:433, 1938.

30. Meyer, K.: Physiol. Rev. **27**:335, 1947.

inactive adjacent supporting stroma. During periods of apparent inactivity lobular stroma and supporting stroma may be morphologically identical. However, comparison with the tissues listed which respond readily to hormones indicates important differences. Cellular changes are variable and follow no apparent pattern. Metachromatic material is found, but the amount is quite variable. Perhaps most important, metachromatic material is most commonly associated with collagenous stroma; we find no evidence of the relationship to fibers, which is a rather consistent feature of the areas tabulated.

However, fibroadenomas are quite similar to connective tissue areas; they respond readily to hormones, as is indicated in the Table. One is led to the conclusion that the lobular stroma of the breast responds in a manner which differs in some details from that of the other areas discussed and that fibroadenomas respond in a manner similar to that of these tissue areas. Hence it follows that lobular stroma and the stroma of fibroadenomas respond differently from each other. Obviously these conclusions must be tested by observations on further material. In view of the meager amount of information available, speculation upon the reasons for the differences found does not seem indicated.

SUMMARY AND CONCLUSIONS

The lobular and periductal stroma of the breast, as well as the stroma of fibroadenomas, have been studied by means of a group of staining and histochemical procedures.

The varieties of lobular stroma which have been described in the literature have been identified. Objections to the sequence of stromal changes associated with the menstrual cycle which appear in the literature are stated.

These tissues differ in some details from connective tissue areas which are particularly responsive to hormones, although they belong to the same general group. On the other hand, the stroma of fibroadenomas responds in a manner quite similar to the connective tissue areas which respond readily to hormones.

Photographs were prepared by Mr. Walter Elle, Barnard Free Skin and Cancer Hospital, and by Mr. Cramer Lewis, Department of Photography, Washington University School of Medicine.

RELATIONSHIP OF ASTEROID BODIES TO LIPIDS

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ASTEROID bodies were apparently first described by Goldmann [1] in 1890. Since that time, numerous examples can be found in the medical literature. The most pertinent articles are those of Wolbach,[2] Hirsch,[3] Cunningham,[4] and Friedman.[5] The reader is referred to Hirsch [3] and Cunningham [4] for a comprehensive review of the literature. Only the material germane to the study at hand will be briefly mentioned here.

The bodies have been variously named "asteroid bodies," "stellate inclusions," and "radial inclusions." They have been described in the lung, lymph nodes, skin and subcutaneous tissue, liver, spleen, breast, uterus, ovaries, epididymis, thyroid, and occasionally in other locations. They have been noted in such diversified conditions as dermoid cysts of ovaries,[6] paraffinomas,[7] lymph nodes draining foci of carcinoma,[8] retention of duct secretion in breast,[9] cryptococcis,[10] leprosy,[11] a probable case of Weber-Christian disease,[12] tuberculosis,[13] sarcoidosis,[14] adenomyoma of uterus,[15] and talcosis.[16]

The asteroid bodies stained preferentially with phosphotungstic acid-hematoxylin.[17] By Fischler's method [13] they reacted as fatty acids, while by the Lor-

From the Department of Pathology, Harvard Medical School, and Peter Bent Brigham Hospital.

1. Goldmann, E. E.: Beitr. path. Anat. **7**:553, 1890.
2. Wolbach, S. B.: J. M. Research **24**:243, 1911.
3. Hirsch, E. F.: Arch. Path. **20**:665, 1935.
4. Cunningham, J. A.: Am. J. Path. **27**:761, 1951.
5. Friedman, M.: Am. J. Path. **20**:621, 1944.
6. Goldmann.[1] Wolbach.[2]
7. (*a*) De Buck, D., and Broeckaert, J.: Bull. Acad. roy. méd. Belgique **17**:890, 1903. (*b*) Hirsch.[3]
8. Symmers, W. St., C.: Am. J. Path. **27**:493, 1951.
9. (*a*) Letulle, M.: Rev. gynec. e Chir. Abd. **19**:401, 1912. (*b*) Hirsch.[3]
10. Stoddard, J. L., and Cutler, E. C.: Torula Infection in Man, Monograph 6, Rockefeller Institute for Medical Research, 1916, p. 55.
11. Mallory, F. B.: The Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1914, pp. 207 and 613.
12. Görl, P.: Arch. Dermat. u. Syph. **148**:130, 1924.
13. (*a*) Herxheimer, G., and Roth, W.: Beitr. path. Anat. **61**:1, 1916. (*b*) Hirsch.[3]
14. Lever, W. F., and Freiman, D. G.: Arch. Dermat. & Syph. **57**:639, 1948. Friedman.[5] Herxheimer and Roth.[13a]
15. Iwanzoff, P.: Beitr. path. Anat. **52**:202, 1912.
16. Jaques, W. E., and Benirschke, K.: A. M. A. Arch. Indust. Hyg. **5**:451, 1952.
17. Wolbach.[2] Hirsch.[3]

raine-Smith-Dietrich stain [18] they reacted as phospholipids. They did not stain by the periodic acid-Schiff method, by von Kossa's method for calcium, or by the Berlin blue method for iron. Tests for elastic tissue were not consistent.[4] The Feulgen test for desoxyribonucleic acid yielded negative results, and the Ziehl-Neelsen stain did not stain the inclusion.[4] They were insoluble in the usual reagents used in fixing, embedding, and staining the tissues (acids and alkalies,[18] alcohol,[5] and xylene [5]). They did not react with the usual stains for fat.[19] They did not contain amyloid, glycogen,[18a] mucin,[19a] or iron [2] and did not reduce silver nitrate.[2] The inclusions were found to disappear between 350 C. and 650 C.[4]

The nature of the bodies has been variously stated to consist of lipid substances,[20] hypertrophied centrosomes or asters,[21] fibrin derivatives,[2] derivatives of elastin,[22] cholesterol,[23] degenerative products of cells,[15] and organic protein structures.[4]

MATERIAL AND METHOD

Tissues from 23 necropsies and 9 surgical specimens comprised the material for this study. Only the tissues containing asteroid bodies were studied in detail at necropsy. The tissues were fixed in Zenker's solution and/or 10% formalin (formaldehyde solution U. S. P. diluted 1 : 10) and stained routinely with hematoxylin and eosin or phloxine-methylene blue. In many instances sections were stained with Mallory's aniline blue, phosphotungstic acid-hematoxylin, Hotchkiss-McManus, Schultz, Feulgen-Bauer, Dunn-Thompson, Smith-Dietrich, Ziehl-Neelsen, and Fischler stains. Tissue from a lymph node of one necropsy was deparaffinized and placed in distilled water, acetone, absolute alcohol, and ethyl ether at room temperature for 48 hours. Similar sections were placed in pyridine and in a mixture of glacial acetic acid and xylene (1 : 1) for 48 hours at 60 C., after which the sections were stained with hematoxylin and eosin.

RESULTS

The morphology of the asteroid bodies presented no new features. Radial inclusions were found in the skin, lungs, lymph nodes, spleen, liver, subcutaneous tissue, breast, and coronary artery.

An attempt is made here to describe the apparent sequences in the genesis of these structures. The earliest form (Fig. 1*A*) consisted 1 to 30 small vacuoles, approximately 1 μ in diameter, containing a central eosinophilic body. At a later stage (Fig. 1*B*) a filamentous eosinophilic process was noted to arise from confluence of these vacuoles. The fully developed stellate inclusion contained 10 to 15 similar filaments and measured 5 to 25 μ in diameter. An eosinophilic spherical body, about 1 μ in diameter, formed the center of the inclusion. Finally, only the asteroid body was found in the giant cells. No asteroid bodies were found in an extracellular location. Several examples of multiple radial inclusions were found

18. (*a*) Vogel, K.: Arch. path. Anat. **206**:157, 1911. (*b*) Wolbach.[2] (*c*) Cunningham.[4] (*d*) Herxheimer, and Roth.[13a] (*e*) Iwanzoff.[15]

19. (*a*) Hummel, E.: Arch. path. Anat. **211**:173, 1913. (*b*) Wolbach.[2] (*c*) Herxheimer, and Roth.[13a] (*d*) Iwanzoff.[15] (*e*) Vogel.[18a]

20. Goldmann.[1] Hirsch.[3] Herxheimer, and Roth.[13a]

21. Kranzfeld, M.: Frankfurt. Ztschr. Path. **15**:297, 1914. De Buck, and Broeckaert.[7a] Iwanzoff.[15]

22. Letulle.[9a] Vogel.[18a] Hummel.[19a]

23. Ernst, P.: Beitr. path. Anat. **53**:429, 1912.

in the giant cells. The giant cells containing asteroid bodies were occasionally associated with granulomata, but numerous examples of single giant cells not associated with granulomata were encountered. In four of the tissue specimens on which necropsy was done, cholesterol clefts were in direct association with the asteroid bodies, and frequently both occupied the same cells.

The bodies generally stained red with hematoxylin and eosin and with phloxine-methylene blue. A few examples of blue staining with these stains were noted, however. A deep red was imparted to these structures with Mallory's analine blue, while they stained a deep blue or purple with phosphotungstic acid-hematoxylin. The bodies did not stain preferentially with the Feulgen-Bauer, Hotchkiss-McManus, Schultz, or Fischler stains. With a few exceptions, the bodies were not acid-fast. Probably the most significant staining reaction occurred with

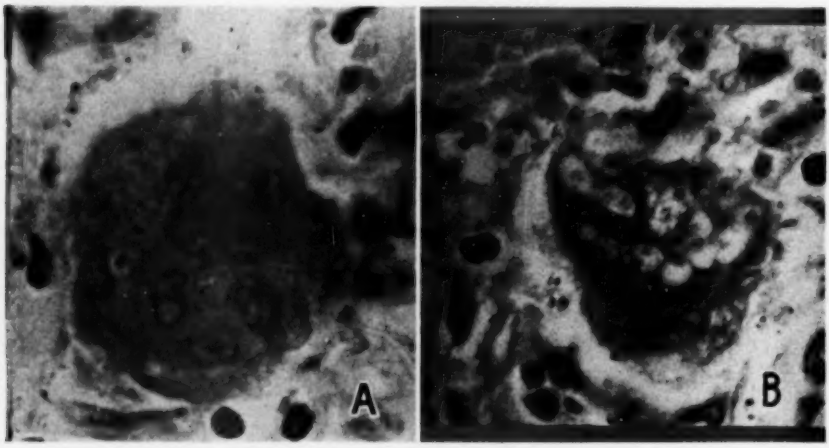

Fig. 1.—*A*, lymph node. Multinucleated giant cell containing approximately 20 clear vacuoles; in each vacuole an eosinophilic center is demonstrated. Hematoxylin and eosin; × 1,000. *B*, lymph node. Multinucleated giant cell showing numerous vacuoles and demonstrating early development of filamentous processes. Hematoxylin and eosin; × 1,000.

the Lorraine-Smith-Dietrich stain for phospholipids. The asteroid bodies stained a deep black in all instances. The central bodies in the vacuoles were observed to stain positively in the early stages.

The inclusions were insoluble in distilled water, absolute ethyl alcohol, acetone, ethyl ether at room temperature, pyridine, and the glacial acetic acid-xylene mixture (60 C. for 48 hours).

The table lists the salient clinical and pathological findings in the cases under consideration.

COMMENT

The cases in which necropsies were done (Table) fall easily into two groups: the endogenous, or diffuse, and the exogenous, or focal. The former is represented by the first 16 cases and differs from the latter group since the endogenous group involves more than one organ and in each section contains asteroid bodies.

Clinical and Pathologic Data in Cases Examined

Case	Age, Yr.	Sex	Organs Involved	Pertinent Clinical and Pathologic Data	Microscopic Appearance
				Autopsy Material	
1	64	F	Lungs	Diabetes mellitus, mesenteric thrombosis	Asteroids in giant cells with no distinct granulomatous tissue; inclusions generalized and frequently intralymphatic
2	72	M	Lymph nodes	Diabetes mellitus, myocardial infarction	As in Case 1
3	67	M	Lungs, lymph nodes	Diabetes mellitus, biliary cirrhosis	As in Case 1
4	73	F	Lungs, spleen, lymph nodes	Diabetes mellitus, intracapillary glomerulosclerosis	As in Case 1
5	55	F	Lymph nodes	Diabetes mellitus, myocardial infarction	As in Case 1; a few granulomata in liver without inclusions
6	57	F	Lungs	Diabetes mellitus, myocardial infarct	As in Case 1
7	77	F	Lungs	Diabetes mellitus, myocardial infarct	As in Case 1
8	65	F	Lungs, lymph nodes	Diabetes mellitus, pernicious anemia, portal cirrhosis	As in Case 1
9	50	F	Lungs	Chromophobadenoma of hypophysis; fasting blood sugar 212-242 mg./100 ml.	Numerous granulomata in interstitial tissue of lungs with giant cells and radial inclusions
10	50	F	Lungs	Pernicious anemia, pulmonary embolus	As in Case 1
11	50	F	Lungs	Pernicious anemia, amyloidosis	As in Case 1
12	50	M	Lungs, spleen	Cerebral hemorrhage, hyalinization of islands	Focal granulomata containing vacuoles and asteroids frequently intralymphatic
13	52	F	Lungs	Dissecting aneurysm	Scattered giant cells with an occasional inclusion which appears to be intralymphatic
14	64	F	Lungs, lymph nodes	Myocardial infarct, sarcoid-like lesions	Very numerous intracellular inclusions showing all stages of development; granulomata noted; probable parasite noted in lymph node
15	75	M	Lungs	Myocardial infarct	Focal granulomata containing rare asteroid body; questionable parasite noted intravascularly in one section
16	69	F	Lungs	Mesenteric thrombosis; died 10 hr. after admission	Numerous granulomata containing rare asteroid bodies; generally perivascular and occasionally intralymphatic
17	82	F	Lungs	Lipoid pneumonia, mesenteric thrombosis	Focal aggregates of giant cells and a few asteroids in atelectatic portions of lungs; intra-alveolar in location; associated with cholesterol cleft
18	45	M	Lungs	Lipoid pneumonia, lobar pneumonia	As in Case 17
19	81	M	Lungs	Lipoid pneumonia, perforated gastric ulcer	Numerous kipophages in the alveoli; rare radial inclusion in giant cells in these areas
20	83	F	Lungs	Lipoid pneumonia, carcinoma of stomach	As in Case 17
21	76	F	Lungs	Carcinoma of cervix, aspiration pneumonia	Foreign body reaction to aspirated material with an occasional radial inclusion
22	60	F	Lungs	Infectious hepatitis	Numerous inclusions frequently multiple in cells; occasionally associated with cholesterol clefts
23	58	M	Coronary artery	Myocardial infarct	One asteroid at periphery of atheroma; numerous lipophages and cholesterol clefts associated with giant cells
				Surgical Cases	
1	72	F	Subcutaneous fat	Paraffinoma	Numerous giant cells with rare stellate inclusion; vacuolated cells abundant
2	46	M	Lungs	Pulmonary tuberculosis	Asteroid bodies and vacuolated giant cells frequently associated with cholesterol clefts at the periphery of caseation necrosis
3	37	M	Lungs	Pulmonary tuberculosis	As in Case 2
4	?	F	Lymph nodes	Carcinoma of breast	Sarcoid-like lesions with fairly numerous asteroid bodies
5	?	M	Skin	Cavernous hemangioma	Foreign body reaction with hemosiderin pigment, cholesterol clefts, and radial inclusions
6	52	F	Skin	Erythema induratum	Fat necrosis with few asteroid bodies
7	?	F	Breast	Old breast abscess	Sarcoid-like lesions with numerous asteroid bodies and crystals of apparent lipids
8	59	M	Lymph node	Sarcoidosis	Granulomata with a few radial inclusions
9	61	F	Lymph node	Sarcoidosis	Numerous asteroid bodies in giant cells of granulomata

In the latter group, represented by the last seven cases, the lesions are focal in distribution. There is only one organ involved, and only a few sections of the organ involved contain the lesions.

Pulmonary involvement is present in most cases, but the anatomic location of the lesions differs in the two groups. In the endogenous group, the lesions are found only in the interstitial tissues, especially in the peribronchial area, in the perivascular area, in the interlobular septae, and in the subpleural area. The lesions are generally intralymphatic (Fig. 2), and frequently distinct endothelial cells can be noted lining the space occupied by the giant cell or granuloma. In the exogenous group the lesions are generally intra-alveolar in location and generally found in areas of atelectasis.

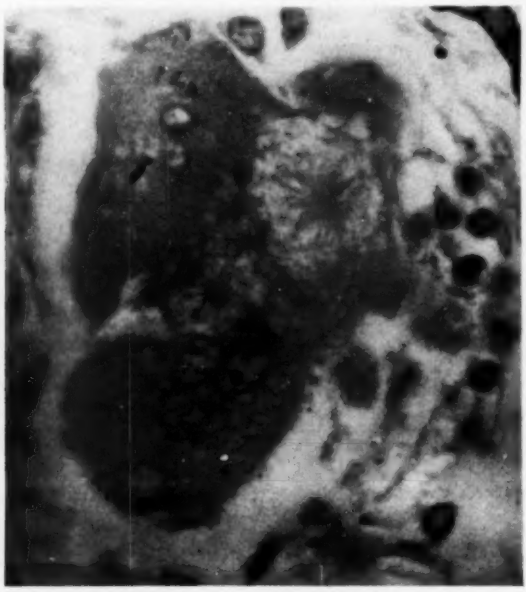

Fig. 2.—Lung. A large multinucleated giant cell containing two fully-developed asteroid bodies and numerous vacuoles. The giant cell is apparently intralymphatic in distribution. Hematoxylin and eosin; × 210.

Twelve patients of the endogenous group displayed clinical evidence of disturbance of lipid metabolism. A glance at the Table shows that the first 8 patients had clinical evidence of diabetes mellitus. Still another patient (Case 12) displayed slight hyalinization of the islets of Langerhans, although no clinical evidence of diabetes mellitus was noted. This represents one-half of the patients of the endogenous group with clinical evidence of diabetes. Two patients demonstrated clinical evidence of pernicious anemia for a number of years, while one of the diabetics also demonstrated evidence of pernicious anemia. In a case of chromophobe adenoma of the hypophysis cerebri, the patient had a fasting blood sugar of 212 to 242 mg. per 100 ml., a diabetic type curve with a glucose tolerance test, and a

blood cholesterol of 303 mg. per 100 ml. The combined cases of diabetes mellitus, pernicious anemia, and chromophobe adenoma account for over two-thirds of the endogenous group. It is a well-known fact that in diabetes mellitus a hyperlipemia is encountered and that in pernicious anemia under therapy with a high reticulocyte count a similar condition exists.[24]

The last four cases of the endogenous group demonstrated no common denominator, although in two cases the patients showed a probable parasite in the lung.

In the seven cases in which necropsies were done, comprising the exogenous group, the lesions are associated with foci of cholesterol deposition and frequently with foci of phagocytosis of fatty substance. It was also noted that the lesions were generally found in the atelectatic portions and especially in the subpleural area.

It is postulated that both groups are due to a disturbance of lipid metabolism. The fault, at least in some cases of the endogenous group, can be said to be hyperlipemia consequent to the diabetes mellitus and pernicious anemia. The incidence of diabetes mellitus in this series (one-half clinically and possibly more if one considers diabetes mellitus to be present in the chromophobe adenoma) far exceeds the general attack rate of diabetes mellitus (i.e., approximately 0.3 to 0.4%). Still in another case the patient displayed hyalinization of the islets of Langerhans, although no clinical diabetes was apparent. It is of interest to note that Hirsch's report of 10 cases in which autopsies were done include a case of diabetes mellitus and a case of pernicious anemia. Wolbach[2] also included a case of pernicious anemia in his five cases. The exogenous group represents a local lipid disturbance usually associated with necrosis of tissue, lipoid pneumonia, or other localized lipid derangements, which will become more obvious on studying the surgical cases. The first 7 surgical cases display a common denominator, i.e., fat necrosis. It was particularly interesting to note that the asteroid bodies were found only near caseous areas and associated with cholesterol clefts in the cases of pulmonary tuberculosis. The last two cases represent cases of sarcoidosis.

The nature of the stellate inclusions would appear to be intimately associated with phospholipids. The positive staining reaction by the Lorraine-Smith-Dietrich method would seem to substantiate this idea. Baker,[25] when studying the Golgi apparatus, stated that the Lorraine-Smith-Dietrich stain was specific for phospholipids. The deep purple staining with phosphotungstic acid-hematoxylin is probable evidence in favor of a phospholipid component. The occasional basophilic staining of these bodies can also be accounted for by a phospholipid component, since these substances are amphoteric. Herxheimer and Roth[13a] noted that the central bodies of the inclusions stained with Lorraine-Smith-Dietrich stain but failed to demonstrate staining of the filamentous processes. The solubility studies are more difficult to assess. According to Baker,[25] phospholipids should be insoluble in absolute ethyl alcohol, acetone, and ethyl ether and soluble in pyrine and a mixture of xylene and glacial acetic acid (1:1). The bodies were found to be insoluble in all these liquids.

24. Kirk, E.: Am. J. M. Sc. **196**:648, 1938.

25. Baker, J. R.: Quart. J. Micr. Sc. **85**:Pt. 1, p. 1, 1944.

It is quite possible that these inclusions represent a lipoprotein. This could account for the staining reactions, since one of the moieties consists of a phospholipid. The peculiar solubilities might represent proteolipids, as described by Folch and Lees.[26]

It should be emphasized that the majority of cases would be considered sarcoid-like lesions and not sarcoidosis.[27] One can only conjecture whether some cases of sarcoidosis represent an altered host capacity to react to a deranged lipoprotein metabolism. Certainly many of the sarcoid-like lesions can be accounted for by a focal or general derangement in lipoproteins, whether endogenous or exogenous.

SUMMARY

Asteroid bodies in tissues from 23 autopsies and 9 surgical specimens were studied for clinicopathological correlation. The cases have been divided into endogenous and exogenous groups. A correlation was made between hyperlipemia, or local destruction of lipids, and the incidence of these lesions, which probably contain lipoprotein as their principal component.

26. Folch, J., and Lees, M.: J. Biol. Chem. **191**:807, 1951.

27. Jaques, W. E.: A. M. A. Arch. Path. **53**:558, 1952.

HYDROPIC CHANGES IN BETA CELLS OF ISLETS OF LANGERHANS

Experimental Hydropic Changes Not Associated with Diabetes Mellitus

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MINNEAPOLIS

SINCE hydropic change of the islets of Langerhans was first described in this country by Allen [1] and Homans,[2] numerous reports have appeared in the literature concerning this change. It has generally been considered a strain phenomenon.[3] As far as can be determined, hydropic change has been described only in permanent diabetes mellitus or during the period when diabetes mellitus is rapidly developing. This change has been considered to be pathognomonic of both human and experimental diabetes mellitus.[3]

We wish to describe relatively simple and consistently successful methods of producing hydropic changes in the beta cells of the islets of Langerhans not associated with diabetes mellitus in the rat.

EXPERIMENTAL METHODS

Three series of experiments were carried out: (*a*) simple starvation followed by a regular diet; (*b*) daily injections of insulin for a period of 14 days, followed by a regular diet; (*c*) same as *b*, except that extra carbohydrate was given in addition to the regular diet. In all instances when the starvation or the insulin was discontinued, a biopsy of tissue from the pancreas was done to determine the structure of the islets with respect to the beta granulation and hydropic changes. The regular diet consisted of Purina fox chow supplemented by a mixture of oats and wheat. The rats were killed at daily intervals, and their pancreases were fixed in 4% formalin and absolute alcohol. The formalin-fixed tissues were stained with our modification of Gömöri's chrome-alum-hematoxylin stain,[4] the trichrome method, the Schiff reagent, and the routine hematoxylin and eosin stain. The alcohol-fixed tissues were stained for glycogen with the Schiff reagent and with Best's carmine stain.

GROUP A.—About 75 rats were starved for 12 days. Water was allowed freely. Only 35 of the 75 rats survived for 12 days. A biopsy of tissue of the pancreas from each of the 35 rats was done before feeding was begun. Twenty rats died as a result of the biopsy, and the 15 survivors were put on the regular diet. Each of the biopsy specimens showed complete

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This work was done under the guidance and with the advice of Dr. E. T. Bell, professor emeritus of the Department of Pathology. The work was supported by a grant from the United States Public Health Service.

1. Allen, F. M.: Studies Concerning Glycosuria and Diabetes, Boston, W. M. Leonard, 1913.

2. Homans, J. H.: (*a*) Degeneration of the Islands of Langerhans Associated with Experimental Diabetes in the Cat, J. M. Research **30**:49, 1914; (*b*) A Study of Experimental Diabetes in the Canine and Its Relation to Human Diabetes, ibid. **33**:1, 1915.

3. Allen.[1] Homans.[2b]

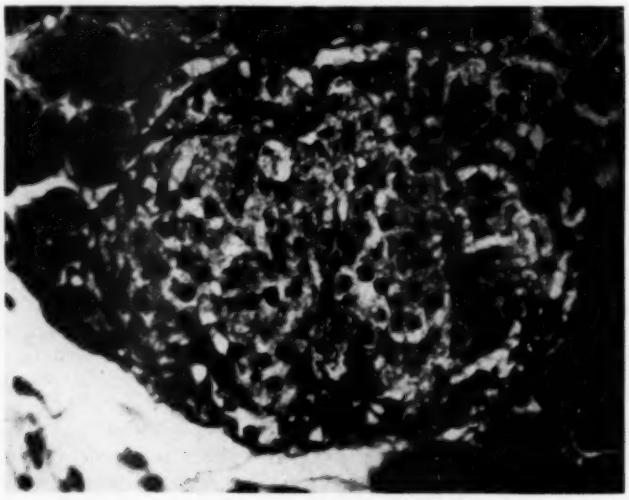
4. Nerenberg, S. T.: To be published.

absence of beta granules and no hydropic degeneration of the beta cells. One rat was killed each day after the feeding was begun.

As regards hydropic changes, this alteration did not develop within the first 24 hours after feeding but reached a maximum within two to four days. After the sixth day the intensity of the hydropic change diminished rapidly, and very little was usually present after the seventh day. None of the rats had glycosuria at any time.

Group B.—The rats of this group were each given four units of protamine zinc insulin parenterally each day for 14 days. Sixteen of the 30 rats of this group died of hypoglycemia before the 14th day. A biopsy of tissue from the pancreas from each of the 14 survivors was done. The biopsy specimens all showed complete absence of beta granules, and a few showed mild hydropic changes in a few beta cells.

One rat was killed each day after the feeding was begun. The development of the hydropic changes was almost identical to those of Group A. The hydropic change appeared after 24



Hydropic changes in the beta cells of an islet of Langerhans of the rat. Third day of regular diet following cessation of administration of insulin. Trichrome stain.

hours of feeding and reached a maximum from the second to the fourth day (Fig.). It receded rapidly after the sixth day. None of the animals had glycosuria.

Group C.—This group of about 100 rats was given insulin for 14 days as in Group B. Tissue from the pancreas was taken for biopsy from each of the 50 survivors. All of the specimens showed complete absence of beta granules with little or no hydropic changes. The survivors were subdivided into three subgroups: Subgroup C_1 consisted of 14 rats. The rats were on the regular diet, and each was given 20 cc. of 10% dextrose subcutaneously twice daily. Subgroup C_2 consisted of 14 rats. The rats were on the regular diet, and each was given 5 cc. of 50% glucose by stomach tube twice daily. Subgroup C_3 consisted of 14 rats. The rats were on the regular diet and were offered a solution of 50% sucrose for drinking. They drank an average of 10 to 15 cc. of this solution daily.

The rats of Subgroups C_1 and C_2 showed no glycosuria at any time, but those of Subgroup C_3 showed a heavy glycosuria for the first 4 days, after which time the glycosuria usually disappeared. Normal control rats given the same amount of glucose by stomach tube do not develop glycosuria.

One animal of each subgroup was killed daily, and the pancreases were studied microscopically. The hydropic change did not appear within the first 24 hours. It reached its maximum on the second day, somewhat earlier than in Groups A and B. It began to recede on the third day and was entirely absent on the fourth and subsequent days.

There is no difference in the maximum intensity of the hydropic change in Groups A, B, and C, but it disappears earlier in Group C.

Hydropic Changes.—Hydropic changes consist of vacuolization of the cytoplasm of the beta cells, the alpha cells being unaffected. The islets are larger than normal. The degree of vacuolization is more pronounced than that obtained by Richardson [5] in dogs, following injections of anterior pituitary extract. The nuclei of the affected beta cells stain normally. The hydropic changes are easily seen in preparations stained with hematoxylin and eosin but are more sharply demonstrated with the trichrome stain. As the beta granules reappear, following feeding after starvation or discontinuance of insulin, they are first seen in the cytoplasm, adjacent to the vacuoles. The vacuoles do not contain glycogen,[6] as judged by the Schiff reagent and Best's carmine stain on tissues fixed in alcohol. The vacuolization is not uniform in the beta cells either in its stage of development or in its disappearance.

Rats in which the beta cells were degranulated by injections of insulin do not acquire hydropic changes when they are subsequently starved. However, if such animals are fed following the period of starvation, hydropic changes develop.

COMMENT

Hydropic change of the beta cells is generally considered to be a "strain" phenomenon.[7] We feel that the experiments described above lend support to this concept. In each of our experiments the beta cells were degranulated, either by starvation or by the use of exogenous insulin, and therefore contained little or no endogenous insulin. When a load of carbohydrate was presented to the pancreas, a "strain" was produced, since no stored insulin was available to meet the demand.

The above experiments demonstrate that neither starvation alone nor insulin alone will produce hydropic changes in the islets. To produce hydropic changes, food or pure carbohydrate must be given to an animal in which the beta cells have been degranulated. This is evidence that carbohydrate given to an animal with little or no insulin in its pancreas causes the hydropic change. The hydropic change in itself is not evidence of a diabetic state, since most of the animals do not exhibit glycosuria, and the process is quickly reversible.

In some forms of experimental permanent diabetes, hydropic changes in the beta cells, which have been regarded as convincing evidence of a diabetic state, are seen [5]; but the above experiments show that hydropic changes may occur independently of diabetes.

Coincidentally with the disappearance of the hydropic changes in the beta cells, the beta granules gradually reappear.

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5. Richardson, K. C.: The Influence of Diabetogenic Anterior Pituitary Extracts on the Islets of Langerhans in Dogs, Proc. Roy. Soc., London, s.B **128**:153, 1940.

6. Toreson, W. E.: Glycogen Infiltration (So-Called Hydropic Degeneration) of the Pancreas in Experimental and Human Diabetes Mellitus, Am. J. Path. **26**:739, 1950.

7. Allen.[1] Homans.[2]

Animals from which the insulin has been largely removed from the pancreas by starvation or by repeated injections of insulin have a decreased carbohydrate tolerance, which may be called "starvation" and "insulin" diabetes respectively. These types of decreased carbohydrate tolerance are not true diabetes mellitus.

SUMMARY

Relatively simple and consistent methods for producing hydropic changes in the beta cells of the islets of Langerhans not associated with diabetes mellitus have been described. The experiments described in this paper lend support to the idea that the hydropic change is a "strain" phenomenon. Hydropic changes in the islets of Langerhans should no longer be considered as pathognomonic of diabetes mellitus in animals.

Case Reports

MUCOEPIDERMOID CARCINOMA OF THE SALIVARY GLAND

Report of a Case with Autopsy Findings

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IT IS ONLY within the last few years that a group of salivary gland tumors have been differentiated from the common salivary tumors, and the term "mucoepidermoid carcinoma" was applied to them by Stewart, Foote, and Becker.[1] These authors concluded that this type constitutes approximately 5% of all salivary gland tumors.

The varying histological pattern from case to case previously had caused considerable confusion, and many of the tumors were called "adenocarcinoma," "cylindroma," or "cystadenoma," the remaining tumors being classified under the heading "mixed parotid tumor."

These tumors may be benign or malignant; in either case, they do not as a rule reach a very large size, and encapsulation may not be seen in the benign type.

The following case of a mucoepidermoid carcinoma is presented because of the fairly rapid growth of the tumor and the widespread metastases which were found at autopsy.

REPORT OF CASE

A 46-year-old unmarried Negro woman was admitted to the hospital on July 22, 1952, with a chief complaint of swellings in the mouth and in the neck. She had been well up to eight months prior to admission, when she started to lose weight. A swelling in the right side of the mouth was noted, followed by a swelling on the right side of the neck and multiple swellings in the skin of the trunk. The tongue became involved, making swallowing difficult and necessitating a fluid diet. Phonation became increasingly difficult and interfered with the patient's offering a history.

There was no known previous hospital admission.

Physical Examination.—Findings on examination were as follows: pulse rate 85 per minute, respiratory rate 20 per minute, temperature 99 F., and blood pressure 100/75. The patient was a slightly built, markedly emaciated, Negro woman in moderate distress. There were enlarged, firm, nontender nodes measuring up to 2.5 cm. present in the neck bilaterally. Some on the right side were attached to the skin.

The tongue was retracted to the right by a firm red tumor involving the right lateral half from tip to root and almost filling the oral cavity. The throat could not be visualized.

There were a few small palpable nodes in the right axilla, none in the inguinal region. There were approximately 20 nodular lesions in the skin of the right upper arm and trunk, those on the trunk being found chiefly on the anterior aspect of the chest, the largest measuring 2 by 0.5 cm. These were considered to represent metastatic lesions. Examination of the chest revealed rales at both bases. The cardiovascular system presented no abnormality.

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Dr. T. J. Curphey, Director of Laboratories, gave advice in the preparation of this paper, and Mr. D. H. Skelton, B.P.A., provided the photomicrographs.

1. Stewart, F. W.; Foote, F. W., and Becker, W. F.: Ann. Surg. **122**:820-844, 1945.

Fig. 1.—Low-power view showing sheets of darkly staining basal cells and larger paler cells in association with mucous secreting cells. Hematoxylin and eosin; × 100.

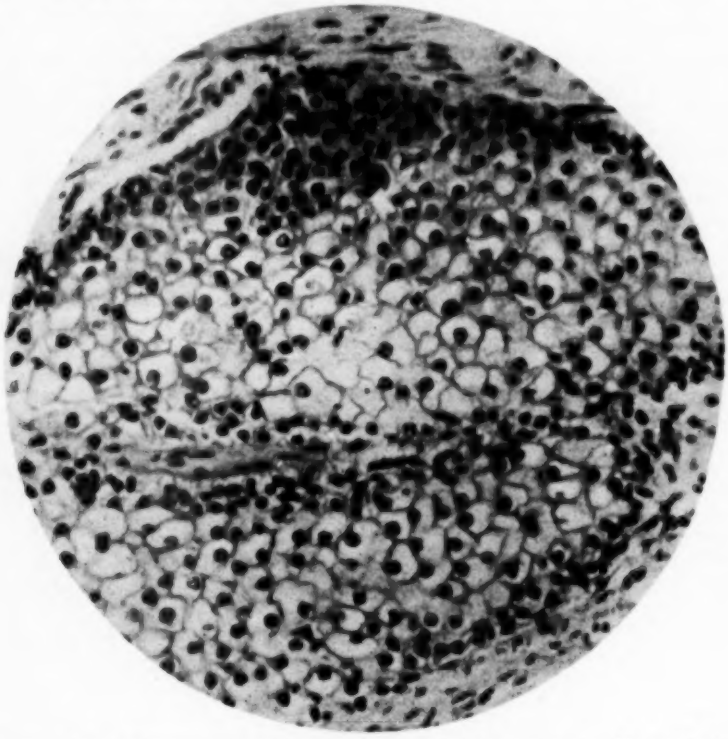

Fig. 2.—Large squamous cells with abundant light-staining cytoplasm. No mucin is present in this section. Hematoxylin and eosin; × 500.

The liver was palpable, firm, and nodular. From the pelvis arose a hard movable mass, the surface of which was nodular. There was marked pitting edema of both lower legs.

A biopsy of tissue of the tongue was performed on the day of admission to the hospital, and the lesion was diagnosed as mucoepidermoid carcinoma of salivary gland origin. The patient's general condition deteriorated, and she died on the third hospital day with signs of bronchopneumonia.

Autopsy was performed approximately 10 hours after death. The external findings were those of the clinical examination. Each pleural cavity contained 250 cc. of serosanguineous fluid. Secondary carcinomatous deposits were present in all the ribs. Removal of the larynx

Fig. 3.—The lumen, as well as occasional tumor cells, contain mucin. Hematoxylin and eosin; × 700.

revealed extension of the tumor into the oral cavity, the larynx, pharynx, and hypopharynx; numerous adjacent lymph nodes showed metastatic carcinoma. There was tumor about the hilar region of the lungs but no evidence of involvement of the lung parenchyma (weight of right lung, 330 gm.; left lung, 400 gm.). The pericardium was studded, chiefly anteriorly, with secondary deposits. Several metastases were present in the left ventricular myocardium (heart weight 200 gm.).

The omentum and mesentery were studded with small carcinomatous seedlings, and secondary deposits also were present in the liver and left adrenal gland.

The mass noted clinically to arise from the pelvis was a uterine fibroid measuring 15 by 10 by 12 cm., the surface of which presented numerous secondary deposits. The genitourinary tract was otherwise not unusual.

Microscopic Examination.—Microscopic examination confirmed the presence of tumor in the floor of the mouth (right side), tongue, pharyngeal wall, larynx, lungs, heart, liver, left adrenal gland, kidneys, cervical lymph nodes, uterine wall, and skin nodules.

The tumor in some areas (Fig. 1) consisted of small densely packed cells with darkly staining nuclei; in other areas there were regular sheets of polyhedral cells which had a greater quantity of cytoplasm and which showed paler staining of cytoplasm and nucleus (Fig. 2). Mucin production, confirmed by mucicarmine stain, was marked in some areas. Fibrous tissue formation was present in and around the sheets of tumor cells.

Some cells around the mucin were arranged in a pattern suggesting gland formation (Fig. 3); in some areas the mucin-producing cells bordering these spaces were columnar.

The nuclei were oval to round, varied mildly to moderately in size, and were commonly vesicular and mildly hyperchromatic in areas. No mitotic figures were seen in any of the sections.

COMMENT

In this type of carcinoma the spread is often confined to local extension with involvement of the cervical lymph nodes, but metastases to the liver occasionally occur.

In Stewart's cases of 19 malignant tumors, 8 showed confirmed distant metastases. However, at the time of his publication, in only one of the eight cases of widespread metastases had an autopsy been held.

An interesting finding in the present case is the numerous distant subcutaneous metastases, which were also present in three of Stewart's cases.

Rawson, Howard, Royster, and Horn [2] reviewed all tumors of the major salivary glands in the files of the Laboratory of Surgical Pathology of the Hospital of the University of Pennsylvania from 1925 to 1948. One hundred sixty cases were studied, and 12 were considered to be mucoepidermoid tumors. Of these, eight were of low-grade malignancy, three of high-grade malignancy, and one of uncertain classification. Of the eight patients whose tumors were relatively benign, all were alive and free from tumor 6 to 16 years after onset, despite local recurrence in six. Of the three patients whose tumors were highly malignant, all were dead within two years after onset, two with widespread local disease and one with metastases.

Recurrence of the tumor following operative removal is not uncommon and usually occurs within a few months of the operation, although in one of Stewart's patients the tumor recurred after an interval of seven years.

The histological pattern of the mucoepidermoid carcinoma may vary considerably. It is now believed that these tumors arise from the salivary gland ducts. The lining epithelium of these ducts contains scattered mucous cells. The other cells which comprise the duct epithelium are basal cells, columnar cells, and rounded or elliptical intermediate cells.

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2. Rawson, A. J.; Howard, J. M.; Royster, H. P., and Horn, R. C.: Cancer **3**:445-458, 1950.

Occasionally there is almost complete overgrowth by epidermoid or squamous cells, with relatively few areas showing mucus secretion.

It is usual, however, to find small sheets of darkly staining basal cells and other sheets of larger polyhedral and pale-staining cells. The degree of mucin production varies and is usually related to the larger paler cells; the histological picture, therefore, may on occasions resemble a basal cell carcinoma in some areas, a transitional cell carcinoma, or a mucous gland adenocarcinoma.

It would seem probable that in certain cases regional lymph node metastases diagnosed as secondary to lymphoepithelioma of the oropharynx, where the primary site is never found, even at autopsy, are, in fact, metastases from a mucoepidermoid carcinoma of salivary gland origin.

Occasionally these tumors may arise in aberrant sites of salivary glands (for example, in the nasopharynx), and it is possible that many of these have previously been diagnosed as epidermoid carcinoma, lymphoepithelioma, or adenocarcinoma.

SUMMARY

A case of mucoepidermoid carcinoma of the right submaxillary gland with numerous widespread metastases is described.

General Reviews

HISTIOCYTOSIS X

Integration of Eosinophilic Granuloma of Bone, "Letterer-Siwe Disease," and "Schüller-Christian Disease" as Related Manifestations of a Single Nosologic Entity

LOUIS LICHTENSTEIN, M.D.
LOS ANGELES

AS A RESULT of the observations of Flori and Parenti,[1] Glanzmann[2] and Wallgren,[3] as well as of Farber,[4] Mallory,[5] Jaffe and Lichtenstein,[6] and Engelbreth-Holm, Teilum, and Christensen,[7] among others, there emerged by 1944 a provisional new concept of the conditions previously designated eosinophilic granuloma of bone, Letterer-Siwe disease, and Schüller-Christian disease as interrelated expressions of the same malady. The European investigators mentioned are to be credited with discerning the intimate relationship between Letterer-Siwe disease so-called (or its numerous equivalent designations) and Schüller-Christian disease, considered in its broad sense without special reference to the Christian triad. The American pathologists cited—and Farber in particular sensed this quickly—were among the first to appreciate the fuller significance of the lesion of eosinophilic granuloma of bone. Specifically, it was realized before long that eosinophilic granuloma may be encountered not only as a skeletel lesion per se [8] but also as a destructive skeletal focus developing in the clinical course of either the Letterer-

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1. Flori, A. G., and Parenti, G. C.: Hyperplastic Infectious Reticulo-Endotheliosis with Granulo-Xanthomatous Evolution (of the Type of Hand-Schüller-Christian), Riv. clin. pediat. **35**:193, 1937.

2. Glanzmann, E.: Infektiose Retikuloendotheliose (Abt-Letterer-Siwe'sche Krankheit) und ihre Beziehungen zum Morbus Schüller-Christian, Ann. pædiat. **155**:1, 1940.

3. Wallgren, A.: Systemic Reticuloendothelial Granuloma: Nonlipoid Reticuloendotheliosis and Schüller-Christian Disease, Am. J. Dis. Child. **60**:471, 1940.

4. (*a*) Farber, S.: The Nature of "Solitary or Eosinophilic Granuloma" of Bone, Am. J. Path. **17**:625, 1941. (*b*) Green, W. T., and Farber, S.: "Eosinophilic or Solitary Granuloma" of Bone, J. Bone & Joint Surg. **24**:499, 1942. (*c*) Farber, S.: The Nature of Some Diseases Ascribed to Disorders of Lipid Metabolism, Am. J. Dis. Child. **68**:350, 1944.

5. Mallory, T. B.: Pathology: Diseases of Bone, New England J. Med. **227**:955, 1942.

6. (*a*) Jaffe, H. L., and Lichtenstein, L.: Eosinophilic Granuloma of Bone: A condition Affecting One, Several or Many Bones, but Apparently Limited to the Skeleton, and Representing the Mildest Clinical Expression of the Peculiar Inflammatory Histiocytosis Also Underlying Letterer-Siwe Disease and Schüller-Christian Disease, Arch. Path. **37**:99, 1944. (*b*) Lichtenstein, L., and Jaffe, H. L.: Eosinophilic Granuloma of Bone, with Report of Case, Am. J. Path. **16**:595, 1940.

7. Engelbreth-Holm, J.; Teilum, G., and Christensen, E.: Eosinophilic Granuloma of Bone—Schüller-Christian Disease, Acta med. scandinav. **118**:292, 1944.

8. Otani, S., and Ehrlich, J.: Solitary Granuloma of Bone, Simulating Primary Neoplasm, Am. J. Path. **16**:479, 1940. Lichtenstein and Jaffe.[6b]

Siwe or the Schüller-Christian syndrome. From correlation of these observations the inference was drawn [6a] that the pathologic common denominator, so to speak, of all three conditions is a distinctive and apparently specific inflammatory histiocytosis, whatever its etiologic agent may ultimately prove to be. Tentative schematizations of the interrelationships predicted upon pathologic data available at that time may be found in papers by Mallory [5] (1942) and by Jaffe and me [6a] (1944). These are still useful for basic orientation, although some of the views expressed need to be revised in the light of recent observations which will be cited presently (Table 1).

The formulation of this integrated concept stimulated the publication of an appreciable number of significant cases whose nature might otherwise have remained more or less obscure. These contributions, many of them comparatively recent, have confirmed beyond any reasonable doubt the essential soundness of the idea that the conditions mentioned represent closely related expressions of a single

TABLE 1.—*Histiocytosis X*

Distribution of Lesions		Clinical Expressions	Age Incidence	Treatment	Prognosis
Localized in bone (one, several, or many foci); no discernible visceral involvement		Eosinophilic granuloma of bone	Infants, children, and young adults (occasionally older adults)	Curettement or x-ray therapy	Cure (although additional skeletal lesions may sometimes appear)
Disseminated	Acute or subacute course	Letterer-Siwe syndrome	Infants and young children below age of 3 years; occasional young adults (adult counterpart of L-S syndrome)	As yet nonspecific: Supportive—Antibiotics for secondary infections; x-ray therapy for skeletal and cutaneous lesions	Serious, though probably not invariably fatal; in occasional children, disease may become chronic or go into remission
	Subchronic or chronic course	Schüller-Christian syndrome (not necessarily Christian triad)	Children and young adults; occasional older adults	As for L-S syndrome; also x-ray therapy or β-hypophamine for diabetes insipidus; x-ray therapy for early pulmonary infiltration; cortisone?	Guarded, especially for children showing active progression and for patients (adults included) with pulmonary fibrosis and/or pituitary involvement

nosologic entity. Some of these papers, moreover, have brought forth important new observations which have served to fill in the major gaps in our still limited fund of essential clinical and pathologic data. In particular, there is now available considerable information in regard to the early phase of the evolution of Schüller-Christian disease so-called, gleaned from biopsy rather than autopsy material, and these findings, which will be discussed later in this article, have forged a stronger link between eosinophilic granuloma and what is commonly called Schüller-Christian disease. Furthermore, it now appears clearer than ever that so-called Letterer-Siwe disease and Schüller-Christian disease represent acute (or subacute) and chronic forms respectively of the same systemic malady, as Wallgren [3] cogently postulated in 1940. In fact, it may be difficult in some individual cases to be certain where the subacute form ends and the subchronic begins, but this is a problem that one has to contend with in many diseases and the distinction is essentially an arbitrary one.

Valuable as the data in these papers have been, the significance of them has been somewhat obscured by mounting confusion in nomenclature. Many of the recent contributors to the subject have felt impelled, unfortunately, to devise their own

terminology and to reinterpret the previous observations of others by way of surveying the literature, often on the basis of personal experience with but a single case or two. While this tendency is quite understandable and was perhaps inevitable during a phase of rapid advance and changing ideas, the pertinent literature is fast becoming a veritable Babel as a consequence. It reflects, on the one hand, a striving for nomenclature adequate to characterize hitherto uncharted and hence "atypical" clinical and pathologic observations and, on the other, preoccupation with tracing transitions between rigid disease categories, as expressed by currently employed designations. One may cite a representative subtitle[9] such as "A Case of Eosinophilic Granuloma of Bone Associated with Nonlipid Reticulosis of Skin and Oral Mucosa Under the Clinical Picture of Hand-Schüller-Christian Disease" to indicate why the inexpert reader is rather likely to be perplexed.

It seems to me that the situation at present clearly calls for taking stock, as it were, and for comprehensive reorientation on the basis of the accrued clinical and pathological data. This should not only take full cognizance of significant new findings but must also, if it is to accomplish its purpose, be intrepid enough in the matter of nosology to break away from slavish adherence to the eponyms of Letterer-Siwe disease and Schüller-Christian disease, at least as primary designations. It must be recognized that both these eponyms were adopted as convenient provisional designations for ostensibly finite clinical syndromes at a time when their intimate relationship could hardly be suspected. In other ways also our concept of the conditions so designated has broadened in scope far beyond anything the authors concerned could ever envision. By the same token, considerably more is now known concerning the incidence and significance of the lesion we called eosinophilic granuloma than could possibly have been surmised even as recently as 1944, and the appropriate place of this manifestation also in relation to the entire disease complex must be reappraised. It is the main purpose of this paper to present for consideration a revised working hypothesis along these lines, which may serve as a guide pending discovery of the etiologic agent and as a framework of reference within which further relevant observations may be charted.

NOMENCLATURE

As emphasized recently by Pinkus and his associates[9] and by Dennis and Rosahn,[10] some appropriate broad general designation for the malady as a whole, which is applicable to any or all of its manifestations and still is relatively brief and simple, is urgently needed for convenient reference and to obviate further confusion. The recognized types of clinical involvement could be grouped under this general heading and still be differentiated from one another, so that useful distinctions having a bearing on treatment and prognosis are maintained. In the absence of any positive knowledge in regard to specific etiology beyond a suspicion of some as yet unrecognized infection, any such provisional designation must of necessity be a descriptive one expressed in terms of the essential pathologic changes characterizing the condition.

9. Pinkus, H.; Copps, L. A.; Custer, S., and Epstein, S.: Reticulogranuloma: Report of a Case of Eosinophilic Granuloma of Bone Associated with Nonlipid Reticulosis of Skin and Oral Mucosa Under the Clinical Picture of Hand-Schüller-Christian Disease, Am. J. Dis. Child. **77**:503, 1949.

10. Dennis, J. W., and Rosahn, P. D.: The Primary Reticulo-Endothelial Granulomas with Report of an Atypical Case of Letterer-Siwe's Disease, Am. J. Path. **27**:627, 1951.

An appreciable number of such names have already been proposed, although none of these is altogether satisfactory in my opinion. In particular, the designations employing reticuloendothelial hyperplasia [11] or reticuloendotheliosis [12] as their central theme are objectionable on two counts. First, the proliferating cells in question, though apparently derived largely from reticuloendothelial cells (mainly the adventitial reticular cells of blood vessels), are already clearly differentiating as histiocytes displaying phagocytic activity. Moreover, reticuloendotheliosis is a generic term covering proliferation of reticuloendothelial cells due to whatever cause and, as such, may have reference to neoplasia and response to abnormal lipid storage, as well as inflammatory hyperplasia of many types. The designations employing reticulogranuloma [9] and reticuloendothelial granuloma (or granulomatosis), sometimes qualified as systemic [3] or primary,[10] are somewhat better from the fact that they at least convey the impression of an inflammatory response, although they also lack specificity in that they could be applied with as much justification to tuberculosis, typhoid, or brucellosis, for example, as to the condition under discussion. The same dissent may be expressed in regard to the suggestion of histiocytic granulomatosis.[4c] The designation of "eosinophilic xanthomatous granuloma" proposed by Thannhauser [13] seeks to overcome this objection by referring to specific features of the pathologic reaction. Unfortunately, this composite designation does not apply with accuracy to any of its component lesions. Specifically, the lesion of eosinophilic granuloma can hardly be characterized as xanthomatous, while the lesions encountered in the Letterer-Siwe and Schüller-Christian syndromes (apart from any which may have the cytologic character of eosinophilic granuloma) contain no more than a scattering of eosinophiles, among other inflammatory cells. Moreover, the lipid content of such systemic lesions is likely to be minimal or negligible if the condition is not already in an advanced or terminal stage of its evolution.

If we did not propose an acceptable name for the malady as a whole in 1944, beyond designating it as a "peculiar inflammatory histiocytosis," [6a] it was precisely because we were stymied by these very obstacles. It does appear, nevertheless, that some designation built around histiocytosis is appropriate, since this term has the connotation usually of an inflammatory proliferative reaction and it is the one feature common to all the various pathologic expressions of the disease (whether or not these lesions also present a concomitant conspicuous content of eosinophiles and whether or not they show secondarily significant uptake of lipid by the histiocytes). The possibility of "idiopathic histiocytosis" suggested itself, but this name again lacks specificity in that it might apply as well to lesions of sarcoidosis, for example. I can devise no better name at present than "histiocytosis X" for specific reference

11. Gross, P., and Jacox, H. W.: Eosinophilic Granuloma and Certain Other Reticulo-Endothelial Hyperplasias of Bone, Am. J. M. Sc. **203**:673, 1942.

12. (*a*) Imler, A. E.: Reticulo-Endotheliosis, with Report of 2 Cases, Am. J. Roentgenol. **56**:343, 1946. (*b*) Hodgson, J. R.; Kennedy, R. L. J., and Camp, J. D.: Reticulo-Endotheliosis (Hand-Schüller-Christian Disease), Radiology **57**:642, 1951. (*c*) Childs, D. S., Jr., and Kennedy, R. L. J.: Reticulo-Endotheliosis of Children: Treatment with Roentgen Rays, ibid **57**:653, 1951.

13. Thannhauser, S. J.: Eosinophilic Xanthomatous Granuloma Synonymous with Schüller-Christian Syndrome, Essential Xanthomatosis of the Normocholesterolemic Type, Lipid Granuloma, Eosinophilic Granuloma, in Christian, H. A., and Mackenzie, J.: Oxford Medicine, New York, Oxford University Press, 1949, Vol. 4, Pt. 2, Chap. 7A, pp. 343-433.

to the disease complex under discussion. It has the advantage of brevity and, by implication, emphasizes the necessity for an intensive search for the etiologic agent, which clearly constitutes the next major assignment now that the pathologic anatomy of the malady is beginning to be somewhat better understood. At all events, histiocytosis X will be the general name employed in the ensuing discussion, and this may be appropriately qualified to emphasize the significant peculiarities of any individual case. In this connection, it may be pointed out that the primary designation of eosinophilic granuloma has been applied by some recent authors to cases presenting cutaneous,[14] oral,[15] pulmonary,[16] or other extraskeletal lesions in addition to osseous involvement, although this practice is not in accord with the recommendations made in our 1944 paper. The confusion thus created can be obviated by referring to such cases as instances primarily of chronic disseminated histiocytosis X (S-C), and their noteworthy skeletal and extraskeletal features, whatever these may be, can be readily brought out in the subheadings as indicated in Table 2. Viewed in this light, pertinent cases are "atypical" only if our concept of what is typical remains stereotyped, and similarly, the need to trace

TABLE 2.—*Classification of Histiocytosis X*

Histiocytosis X, localized to bone (eosinophilic granuloma, solitary or multiple)
Histiocytosis X, disseminated, acute or subacute (L-S syndrome)
With destructive skeletal lesions (E. G.)
With transition to chronic phase (S-C)
Histiocytosis, disseminated, chronic (S-C syndrome)
With destructive skeletal lesions (E. G.)
With early extraskeletal lesions (indicate sites) resembling E. G.
With acute or subacute exacerbation (L-S)
With involvement predominantly of bones, lungs, pituitary, and/or brain, skin, mucous membranes (oral, anal, genital), liver or lymph nodes, etc. (in varying combinations, as the case may be)

"transitions" from one disease to another exists only if we persist in thinking in terms of rigid compartments.

CLINICAL CONSIDERATIONS

In this section I will not attempt to discuss all the clinical manifestations of the disease complex but will deal rather with certain general considerations having a bearing mainly on treatment and prognosis. At the outset, for the convenient

14. (*a*) Curtis, A. C., and Cawley, E. P.: Eosinophilic Granuloma of Bone with Cutaneous Manifestations: Report of a Case, Arch. Dermat. & Syph. **55**:810, 1947. (*b*) McCullough, N. B.: Eosinophilic Granuloma with Multiple Osseous and Soft-Tissue Lesions in an Adult, Arch. Int. Med. **88**:243, 1951. (*c*) McCreary, J. H.: Eosinophilic Granuloma with Simultaneous Involvement of Skin and Bone, Arch. Dermat. & Syph. **58**:372, 1948.

15. Shroff, J.: Eosinophilic Granuloma of Bone: Case Report of Eosinophilic Granuloma of Mouth (Jaws, Gums and Palate) with Simultaneous Fistula in Ano, Oral Surg. **1**:256, 1948.

16. (*a*) Weinstein, A.; Francis, H. C., and Sprofkin, B. F.: Eosinophilic Granuloma of Bone: Report of a Case with Multiple Lesions of Bone and Pulmonary Infiltration, Arch. Int. Med. **79**:176, 1947. (*b*) Ackerman, A. J.: Eosinophilic Granuloma of Bones Associated with Involvement of Lungs and Diaphragm, Am. J. Roentgenol. **58**:733, 1947. (*c*) Dickson, D. D.: Eosinophilic Granuloma of Bone with Diffuse Pulmonary Involvement, California Med. **69**:51, 1948. (*d*) Brody, A. J.: Multiple Eosinophilic Granuloma of Bone with Pulmonary Involvement, U. S. Armed Forces M. J. **2**:1669, 1951.

orientation of those who are not conversant with the salient clinical features and the interrelationship of eosinophilic granuloma of bone (E. G.), the Letterer-Siwe syndrome (L-S) and the Schüller-Christian syndrome (S-C), these have been schematically outlined in Table 1. From this Table it may be readily inferred that the clinical picture and prognosis in any individual case are significantly influenced by such factors as the dispersion of the lesions (i. e., whether localized in bone or disseminated), the tempo and severity of the disease (possibly related to host susceptibility), the age of the patient (with certain reservations, as noted), and extensive involvement (or lack of it) of certain crucial organs, particularly the lungs, bone marrow, and pituitary.

In regard to the factor of localization, it is now generally recognized that the mildest and most favorable expression of the disease (histiocytosis X) is represented by cases presenting one, several, or occasionally many destructive foci within the skeleton and otherwise showing neither apparent constitutional indications of illness nor any discernible evidence of cutaneous, pulmonary, hypophyseal, or other extraskeletal involvement. The pathologically descriptive name eosinophilic granuloma of bone still seems an appropriate designation for such instances, which merit special consideration by virtue of their auspicious prognosis. I am inclined to regard this skeletal localization as an indication of successful confinement of the etiologic agent, which may well account for the favorable outcome. One can venture a good prognosis for recovery in such cases, even though an appreciable number of skeletal lesions are present and even though additional skeletal lesions subsequently make their appearance, as occasionally happens. In the matter of clinical diagnosis, it is worth emphasizing again that a focus of eosinophilic granuloma of bone is prone to develop so rapidly and to break through the cortex of the affected bone so readily that prior to biopsy it is often mistaken clinically for a primary malignant tumor, especially Ewing's sarcoma. By the same token, comparable multiple foci appearing in the skeleton of a child, for example, may conceivably give rise to an initial impression of metastatic neuroblastoma. Despite their seemingly ominous behavior, however, it is well known that such lesions heal readily following curettement or roentgen therapy, and it has also been demonstrated [68a] that they may even regress or disappear spontaneously. The debate in the literature as to whether lesions of eosinophilic granuloma if untreated necessarily show secondary lipidization and fibrosis in time, i. e., a tendency toward conversion to so-called lipid granuloma, strikes me as a tempest in a teapot, and it is my impression that the pathologic evidence presented in support of this view relates to lesions of bone which develop in the course of chronic disseminated histiocytosis X (S-C).

It is also important to emphasize that in actual practice it is not always possible on initial examination of a patient, especially a young child, to be certain that skeletal lesions pathologically identified as eosinophilic granuloma do not have their histiocytic counterpart elsewhere, or that such extraskeletal lesions will not manifest themselves subsequently, even though at the time roentgen examination of the chest is negative and physical examination fails to reveal involvement of the skin, mucous membranes, or hemopoietic organs. To be more explicit, inasmuch as the pathologic lesion of eosinophilic granuloma of bone represents the expression of acute skeletal involvement in the disease as a whole (histiocytosis X) whether this be localized or disseminated, it follows that from the roentgenogram of such a lesion or even from a biopsy of it alone one cannot make any reliable forecast as

to prognosis, since this depends largely, as noted, upon whether there is associated visceral involvement and, more particularly, upon the distribution, extent, and severity of these visceral lesions. In certain instances, it may be necessary to reserve judgment and follow the patient closely for a number of months or even for several years. In particular, the clinician must be on the alert for such indications as fatigability, chronic malnutrition, loss of weight or failure to gain weight (in a growing child), slight fever, predisposition to secondary infections, and the frequent appearance of new skeletal defects as harbingers of systemic involvement of potentially serious import. To cite a pertinent instance, I have had occasion to follow the case of a child, 3 years of age, whose initial manifestation of the disease was the development of multiple skeletal lesions, including a number in the calvarium, of the nature of eosinophilic granuloma as demonstrated by biopsy. At the time these first appeared, the child was in satisfactory general condition and careful physical examination revealed no clear indication of extraskeletal involvement, although one had some misgivings about her tendency toward infections, especially otitis media, soreness of the gums, and somewhat capricious appetite. This concern was heightened by the continued appearance of new skeletal defects in spite of adequate roentgen therapy. However, many months elapsed before it became evident that the child was actually suffering from Schüller-Christian disease (chronic disseminated histiocytosis X). Terminally, there was rapid deterioration of her condition and sharp acceleration of the tempo of the disease, as manifested by the appearance of a purpuric cutaneous eruption, hepatosplenomegaly, a multitude of fresh skeletal lesions, and, altogether, the clinical picture of so-called Letterer-Siwe disease (acute or subacute disseminated histiocytosis X). This case is of particular interest in that it exemplifies all the various clinical expressions of the disease in a single patient.

Returning to some other general considerations pertaining to the disease as a whole (histiocytosis X), the impression seems to prevail that the seriousness of the malady is directly related to the age of the patient and, more specifically, that the younger the patient the graver is the outlook. To be sure, the great majority of the severe or fatal cases are observed in infants or young children. On the other hand, it should be pointed out that the age factor alone is not necessarily a reliable guide to prognosis. Thus, cases of relatively mild eosinophilic granuloma of bone (localized histiocytosis X) may be observed on occasion in younger children and even in infants, and I have observed a pertinent instance in an infant only 14 months of age; on the other hand, the prognosis is not necessarily favorable in older patients. There is now evidence to indicate that subacute disseminated histiocytosis X (L-S) does not occur exclusively in infants or young children, but has its adult counterpart. The case with autopsy recently reported by Dennis and Rosahn [1] appears to represent an instance in point, and I have knowledge of several comparable instances (seen in consultation during World War II) which, though not followed to termination, seemed clearly to fall into the same category. With reference to chronic disseminated histiocytosis X (S-C) in adult patients, it is, of course, well known that, while the clinical course is usually protracted over a period of many years, the disease may nevertheless prove fatal ultimately, particularly if extensive damage has been inflicted on the pituitary or if serious pulmonary infiltration and fibrosis have resulted in cor pulmonale and right heart failure, another common sequel.

Apropos of the over-all prognosis in patients presenting the manifestations of Schüller-Christian disease so-called (chronic disseminated histiocytosis X), there appears to be rather wide divergence of opinion. Thus, it has been stated by Gross and Jacox [11] and others (presumably on the basis of pooled material from the literature, not specifically cited or tabulated) that there is a 30% probability of a fatal outcome. On the other hand, Schafer [17] has written that an estimated 30% of the patients with Schüller-Christian disease recover (70% ultimate fatality). Actually, on the basis of such random sampling as the literature affords, without adequate allowance for the great variation in clinical severity from case to case and without specific reference to significant involvement of crucial organs, as noted, it is difficult to arrive at any statistically valid estimate. For every fatal instance which is published, there are probably several others of less serious import which do not find their way into the literature or which, perhaps, are not even recognized clinically. From one of the autopsied cases reported by Chester,[18] for example, it is evident that old sclerotized skeletal lesions of S-C disease may be present for many years without any awareness of them by the patient. Incidentally, this tendency to indiscriminate pooling of material is also reflected in two recent papers by Hodgson, Kennedy, and Camp [12b] and Childs and Kennedy [12c] purporting to show the gratifying effectiveness of roentgen therapy in cases of "reticulo-endotheliosis (H-S-C disease)." In the absence of any critical analysis of their case material along the lines indicated, their results, however encouraging they may be, cannot necessarily be regarded as having general application.

In regard to therapy, it may be pointed out that even in relatively serious, progressive cases presenting diabetes insipidus and/or extensive pulmonary infiltration as their major problem it is often possible by alert and well-conceived clinical management to ward off a fatal outcome for some time and occasionally to induce a remission. Such management is concerned mainly with abatement of skeletal foci through the use of adequate roentgen therapy, general supportive measures, prevention and control of potentially serious infections (especially pulmonary) by the judicious use of antibiotics, and amelioration of diabetes insipidus by the use of β-hypophamine (pitressin) or irradiation. The observations of Blahd, Levy, and Bassett [19] suggest that in serious situations cortisone may be of some value in improving the general condition of the patient, although no valid conclusion can be drawn from experience with a single case. Another significant lead in regard to effective treatment comes from the empirical observation of Imler [12a] and of Weinstein, Francis, and Sprofkin [16a] that roentgen therapy may alleviate respiratory difficulty associated with diffuse pulmonary infiltration, provided that the latter has not already gone on to advanced interstitial fibrosis and intractable emphysema. Unfortunately, it seems not to be generally recognized that diffuse pleural and interstitial pulmonary infiltration, leading eventually to fibrosis, honeycombing of the lungs and episodes of spontaneous pneumothorax, is a rather common hallmark of chronic disseminated histiocytosis X (S-C), at least in adults.[20] Specifically, it

17. Schafer, E. L.: Nonlipid Reticulo-Endotheliosis: Letterer-Siwe's Disease: A Report of 3 Cases, Am. J. Path. **25**:49, 1949.

18. Chester, W.: Ueber Lipoidgranulomatose, Arch. path. Anat. **279**:561, 1930.

19. Blahd, W. H.; Levy, M. S., and Bassett, S. H.: A Case of Hand-Schüller-Christian Syndrome Treated with Cortisone, Ann. Int. Med. **35**:927, 1951.

20. Oswald, N., and Parkinson, T.: Honeycomb lungs, Quart. J. Med. **18**:1, 1949.

is not uncommon for such cases to be regarded initially as instances of sarcoidosis. Thus, in the case of Blahd and his associates cited above the clinical recognition of the condition was delayed for almost four years until a small calvarial defect appeared (eosinophilic granuloma on biopsy), although the roentgen appearance of the pulmonary lesions taken in conjunction with the manifestations of diabetes insipidus which the patient also presented should logically have been regarded as strong presumptive evidence of Schüller-Christian disease, even in the absence of any obvious skeletal defects (Figs. 1 and 2).

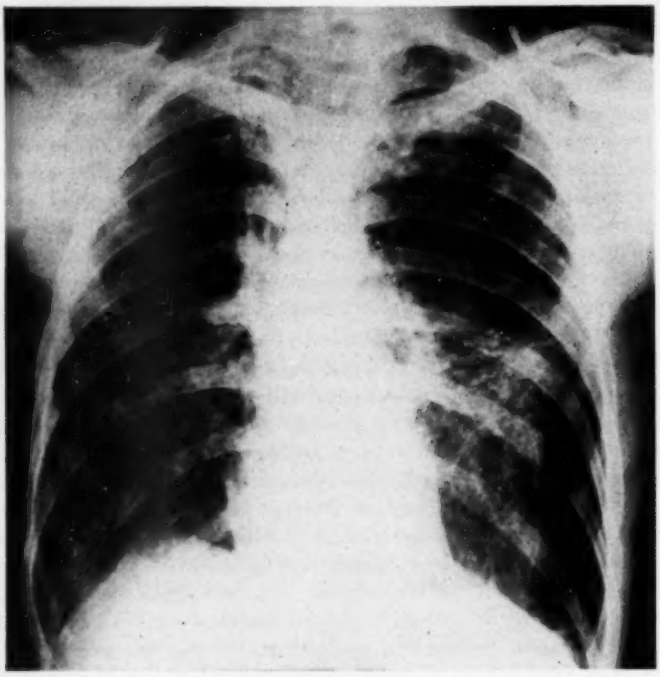

Fig. 1.—Roentgenogram of chest showing rather dense infiltration of both lung fields, suggestive honeycombing, and spontaneous pneumothorax on the left side (below pleural adhesion). The patient was a young man, 29 years of age, who had complained of persistent dry cough and chest pain for several years. This picture, while not pathognomonic, is frequently observed in chronic disseminated histiocytosis X (S-C).

With respect to prognosis in acute or subacute disseminated histiocytosis X (L-S), while this in general is grave, to be sure, there is nevertheless reason to doubt that the outcome is invariably fatal, as is often assumed.[17] There appear to be occasional instances of the Letterer-Siwe syndrome which show a slowing up of their tempo, a tendency toward chronicity, and even clinical remission for several years following sound clinical management and, especially, effective roentgen therapy.[14a] To ascertain the eventual outcome in the relevant instances reported requires longer follow-up than the period of observation to date.

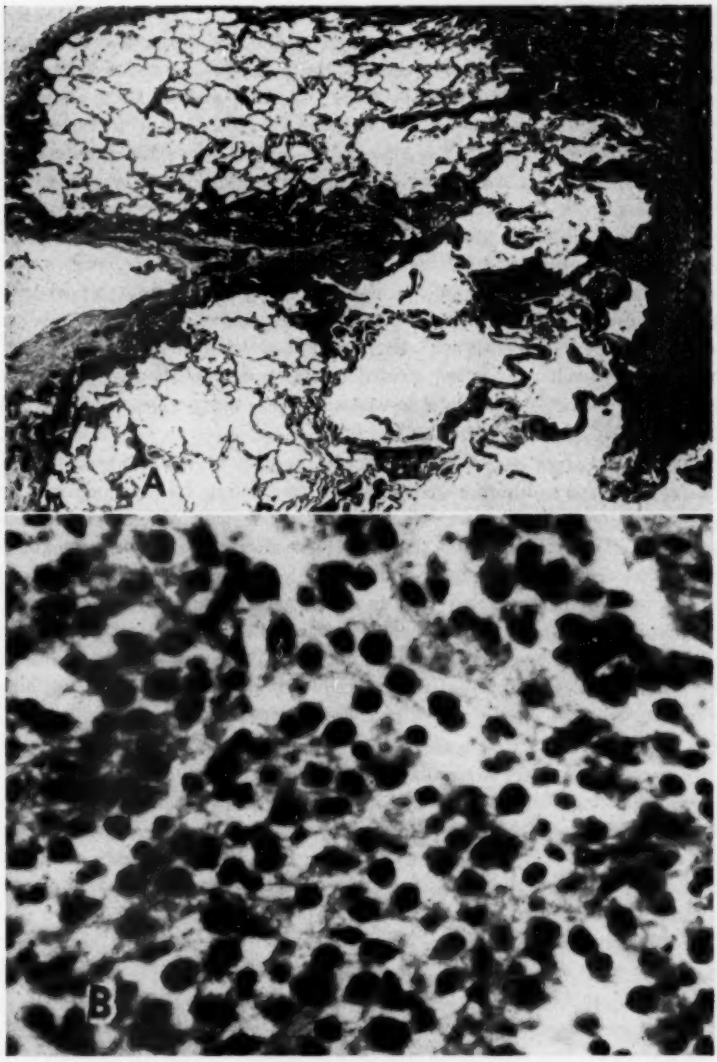

Fig. 2.—*A*, photomicrograph of representative field of lung biopsy obtained by thoracotomy in the case illustrated in Figure 1. The pulmonary parenchyma is split up into emphysematous lobules by tracts of fibrous connective tissue. The latter was infiltrated by numerous small lymphoid follicles. The overlying pleura is distinctly edematous. Within the thickened alveolar septa in places one could discern (at higher magnification) residual nests of histiocytes, some of which had undergone conversion to lipophages; × 22. *B*, photomicrograph of field from calvarial lesion of eosinophilic granuloma from the same case, showing histiocytic cells and occasional leucocytes, along with disintegrating erythrocytes in the background. Other fields showed massed eosinophiles undergoing degeneration and necrosis; × 565.

RECENT PATHOLOGIC OBSERVATIONS OF SIGNIFICANCE

With reference to eosinophilic granuloma of bone, it has already been emphasized that, while the lesion is frequently observed as an apparently isolated skeletal manifestation, it may also develop in the course of disseminated histiocytosis X. That it may do so occasionally in acute or subacute disseminated histiocytosis X (L-S) has been known [6a] for some time. That it often does so in cases of chronic disseminated histiocytosis X (S-C) as either an early or late manifestation, has likewise been established within the last several years [21] and is no longer a matter of conjecture. From the data of a number of these cases, one gathers that in the latter circumstance the lesion of eosinophilic granuloma may retain its original character for many months,[21g] or even as long as a year or more, after its presence is detected, although it also appears that eventually there is a tendency toward fibrosis, diminution in the number of eosinophilic leucocytes present, and conversion of histiocytes to lipophages [22] in the direction of what has been called "lipid granuloma." It seems altogether probable that roentgen therapy accelerates this change. In old burned-out skeletal lesions one also observes reactive osteosclerosis, which apparently persists indefinitely.

Another concept of major importance in regard to the lesion of eosinophilic granuloma has grown out of recent observations noting its presence in a number of extraskeletal sites, as part of the picture apparently of chronic disseminated histiocytosis X. The impression that I gained after analyzing the pertinent case reports is that the lesion of eosinophilic granuloma seems to represent the pathologic expression of early, rather rapidly developing reaction to the etiologic agent, and as such it may appear not only within bone, where its presence was first recognized, but also in other sites as well, notably in lymph nodes, the skin, the oral cavity (gingiva, palate, etc.) and the anogenital region, as well as in the lungs and possibly other viscera (if the whole picture were to be revealed).

The significance of these extraskeletal lesions resembling eosinophilic granuloma warrants their careful annotation. They were first detected, as one might expect, in superficial mucocutaneous sites readily accessible to biopsy.[23] Thus, in the important case reported by Curtis and Cawley,[14a] that of a female infant presenting a reddish papular cutaneous eruption, whitish macules over the palate and tongue, and weeping erosions in the axillary, genital, and perianal regions, biopsies of a number of these sites showed diffuse histiocytic proliferation and in places "a great many eosinophiles" as well. This infant also presented a number of destructive skeletal lesions (in a rib, the left iliac bone, and the right mastoid), as well as some palpable lymph nodes in the cervical and inguinal regions, although biopsy was not done on these. It is relevant to note further that following roentgen therapy there was gradual and eventually complete healing of both the skeletal and the

21. (*a*) Pinkus, Copps, Custer, and Epstein.[9] (*b*) Imler.[12a] (*c*) McCullough.[14b] (*d*) Weinstein, Francis, and Sprofkin.[16a] (*e*) Dickson.[16c] (*f*) Blahd, Levy, and Bassett.[19] (*g*) Currens, J. H., and Popp, W. C.: Xanthomatosis-Hand-Schüller-Christian Type: Report of a Case with Pulmonary Fibrosis, Am. J. M. Sc. **205**:780, 1943. (*h*) Love, F. M., and Fashena, G. J.: Eosinophilic Granuloma of Bone and Hand-Schüller-Christian Disease, J. Pediat. **32**:46, 1948.

22. Versiani, O.; Figueiro, J. M., and Junqueira, M. A.: Hand-Schüller-Christian's Syndrome and "Eosinophilic or Solitary Granuloma of Bone," Am. J. M. Sc. **207**:161, 1944.

23. Footnotes 9, 14, and 15. Thannhauser, S. J.: Eosinophilic Granuloma of Bone: Letter to the Editor, J. A. M. A. **134**:1437, 1947.

cutaneous lesions and that the child has remained well since 1948, no new lesions having appeared to date. Also in the remarkable case reported by McCreary,[14c] that of a child of 11 years, the initial manifestations of the disease at the age of 2 years were tender tumefactions and ulcerations of the skin of the chest, back, and scalp, and enlarged lymph nodes in the submaxillary, cervical, and axillary regions, which subsequently drained. It is interesting to note that fever accompanied the appearance of new lesions. According to the record, furthermore, there was a tendency toward slow spontaneous healing and this was accelerated by roentgen therapy, which afforded remission from time to time for as long as a year. A defect in a parietal bone also developed during the course of the child's illness, but at no time did she manifest either exophthalmus or diabetes insipidus. Biopsies of the lesions in the skin and lymph nodes, as well as in the calvarium, are said to have shown a picture not unlike that of eosinophilic granuloma, although some of the histiocytes present apparently did contain lipid.

Continuing in the same vein, another informative report is that of Pinkus and his associates[9] carefully describing the case of a 9-month-old girl who presented a lesion of eosinophilic granuloma in the calvarium, associated with seborrheic dermatitis of the scalp, chest, and abdomen, as well as an ulcerative lesion of the hard palate and contiguous gingiva. Biopsy of the latter lesion showed a picture that was also labeled eosinophilic granuloma, while pathologic examination of the cutaneous lesions showed conspicuous proliferation of histiocytes, which were not foamy, and otherwise a sprinkling of lymphocytes and eosinophilic granulocytes. It is interesting to note that one and one-half years later, another skeletal lesion appeared in the shaft of a humerus. This child likewise responded satisfactorily to roentgen therapy and is ostensibly well at present, at the age of 6 years. Of comparable import is the case reported by Shroff,[15] that of a man of 51, who presented, in addition to a large destructive lesion in the ramus of the mandible apparently of the nature of eosinophilic granuloma, lesions in the oral cavity (gingiva and palate), as well as a persistent fistula-in-ano. Biopsies of these extraskeletal lesions again showed a picture which was interpreted as being very suggestive of eosinophilic granuloma. Still another revealing report along the same lines, that of McCullough,[14b] concerns a young woman, whose initial manifestation of the disease (chronic disseminated histiocytosis X), at the age of 19, was the appearance of recurring gingival ulcerations, associated with loosening of teeth and rarefaction in the mandible. Somewhat later, a lesion in the frontal bone appeared, followed by another in the lamina of the fifth cervical vertebra. Subsequently, superficial weeping ulcers (described as ragged, with indurated, though undermined, edges) appeared on the labia majora, on the perineum, around the anus, and also within the anal orifice. Still later, another tender lesion was detected in a rib and the patient at this time complained of increasing fatigue and loss of weight (15 lb. [6.8 kg.]). The rib lesion in this case was identified on biopsy as one of eosinophilic granuloma; the perineal ulcers are said to have shown histiocytic proliferation along with some eosinophiles, while the gingival lesion was frankly labeled eosinophilic granuloma. In this patient diabetes insipidus eventually developed, but roentgen therapy was effective in lowering the β-hypophamine requirement and also induced successful healing of the oral and anogenital lesions. At the time of publication she had had remission of symptoms for one and one-half years, after six years or

more of illness. Needless to say, long range follow-up of key cases such as these will be of great value in eventually elucidating the full clinical picture of the malady under discussion.

With reference to the mucocutaneous lesions resembling eosinophilic granuloma which were described in the cases cited above, it may be helpful to those not versed in the dermatologic literature to indicate that these apparently represent one or another expression of disseminated histiocytosis X and, as such, are not to be confused with cutaneous granulomas of nondescript nature which are confined to the skin (of the face, for example) and which may also contain eosinophiles for whatever reason. Such experienced observers as Weidman[24] and Pinkus[25] have expressed the opinion, with which I am wholly in accord, that the latter lesions, though sometimes labeled eosinophilic granuloma, have nothing in common with eosinophilic granuloma as discussed in this paper.

Involvement of lymph nodes by what may also be called eosinophilic granuloma was noted relatively early by Mallory.[5] Through the courtesy of Dr. Alvin G. Foord, I likewise have had occasion to observe a comparable lesion in a cervical node. It is also pertinent to recall that in the case reported by McCreary,[14c] previously cited, there were similarly involved draining lymph nodes which appeared in association with ulcerated cutaneous lesions. Still another well-documented case in point is that of Love and Fashena,[21b] concerning a 19-month-old child with a tumor-like swelling of the mandible; this was resected and along with it a cervical node showing, as did the skeletal lesion, histiocytic proliferation and striking infiltration by eosinophiles. This child, at the age of 4 years, manifested symptoms of diabetes insipidus (which responded to β-hypophamine), and the following year, biopsy was done on two additional cervical lymph nodes, which again showed a picture interpreted as eosinophilic granuloma.

The nature of early visceral lesions in this disease (disseminated histiocytosis X) cannot be as readily ascertained, obviously, as that of lesions appearing in accessible lymph nodes, the oral cavity, the anogenital region, or the skin generally. The lungs, however, do lend themselves to investigation, inasmuch as their involvement may be readily detected by roentgen examination. That roentgenographically discernible, diffuse, interstitial bilateral pulmonary infiltration may develop in patients who also present one or more skeletal lesions of the nature of eosinophilic granuloma, or in whom subsequently such skeletal lesions develop, is now well established by the reports of Currens and Popp,[21g] Imler,[12a] Troxler and Niemetz,[26] Arnold,[27] Weinstein and co-workers,[16a] Ackerman,[16b] Dickson,[16c] Schuknecht and Perlman[28] (Case 1), Brody,[16d] and Blahd and his associates.[19] Incidentally, in at

24. Weidman, F. D.: The "Eosinophilic Granulomas" of the Skin, Arch. Dermat. & Syph. **55**:155, 1947.

25. Cobane, J. H.; Straith, C. L., and Pinkus, H.: Facial Granulomas with Eosinophilia. Their Relation to Other Eosinophilic Granulomas of the Skin and to Reticulogranuloma, Arch. Dermat. & Syph. **61**:442, 1950.

26. Troxler, E. R, and Niemetz, D.: Generalized Xanthomatosis with Pulmonary, Skeletal and Cerebral Manifestations: Report of a Case, Ann. Int. Med. **25**:960, 1946.

27. Arnold, H. L., Sr.: Eosinophilic Granuloma of Bone; Preliminary Report of a Case Complicated by Lung Lesions, Proc. Staff Meet. Clin., Honolulu **12**:183, 1946.

28. Schuknecht, H. F., and Perlman, H. B.: Hand-Schüller-Christian Disease and Eosinophilic Granuloma of the Skull, Ann. Otol. Rhin. & Laryng. **57**:643, 1948.

least two of these cases[29] diabetes insipidus was also present (without apparent calvarial involvement) and in another,[16b] roentgenographic studies indicated that the diaphragm was involved, as well as the lungs.

Until recently, our knowledge concerning the pathologic nature of these pulmonary changes commonly observed in chronic disseminated histiocytosis X (S-C) was limited to the relatively late, or end, stage of their development, as seen in autopsy material. At this late stage, as previously noted, the picture is dominated by pleural and interstitial fibrosis and its sequelae, and the original lesion has already undergone profound secondary change and involution (Fig. 2*A*). From the finding of small nests of lipophages within the connective tissue of such advanced lesions, one is by no means justified in inferring that these pulmonary lesions initially had the character of what may be called "lipid granuloma." Quite the contrary view is suggested by the well-established fact that in infants and young children who succumb quickly to acute disseminated histiocytosis X (L-S) the essential pulmonary change is one of diffuse histiocytic infiltration, comparable to that observed in the viscera generally. Another valuable lead is afforded by the pulmonary findings in the autopsied case reported by Dennis and Rosahn,[10] of an adult, 32 years of age, presenting what may be plausibly interpreted as subacute disseminated histiocytosis X (L-S) with predominant pulmonary involvement. This subject's respiratory difficulty dated back approximately 15 months prior to his demise. In this instance also, the primary change found in the lungs was widespread histiocytic proliferation and infiltration in the pleural, peribronchial, and interstitial tissues, although this had already led to fibrosis and "cystic" honeycombing of the pulmonary parenchyma.[20]

An extraordinary opportunity to gain insight into the character of these pulmonary lesions at an even earlier stage of their development was afforded by the recent paper of Farinacci, Jeffrey, and Lackey,[30] entitled "Eosinophilic Granuloma of the Lung" and dealing with the findings in two patients with pulmonary symptoms of relatively short duration, in whom exploratory thoracotomy and biopsy had been done. Neither of these patients presented concomitant skeletal defects, but, as I have already observed, these may subsequently appear, as may also diabetes insipidus and still other manifestations of the disease (disseminated histiocytosis X). The first of these patients, a 32-year-old man, had complained of productive cough, loss of weight, fatigue, and sweats of several months' duration, and because of this history was admitted to the hospital with a provisional diagnosis of tuberculosis. The second patient, a 24-year-old man, had also complained of a slightly productive cough and some loss of weight during the preceding eight months. In addition, he presented an elevated eosinophile count (10%) and a palpable axillary lymph node, which, however, had not been subjected to biopsy. In both patients roentgenograms of the chest revealed diffuse infiltration of the lung fields, resembling that seen in disseminated histiocytosis X. In keeping with this picture, inspection of the lungs at the time of thoracotomy revealed the presence of small, fibrous, buckshot-like nodules in the pleura, and these were also palpable throughout the parenchyma. Sections of these nodules showed a peculiar granulomatous reaction featured essentially by the presence of numerous large histiocytic cells intermingled

29. Blahd, Levy, and Bassett.[19] Troxler and Niemetz.[26]

30. Farinacci, C. J.; Jeffrey, H. C., and Lackey, R. W.: Eosinophilic Granuloma of the Lung: Report of 2 Cases, U. S. Armed Forces M. J. **2**:1085, 1951.

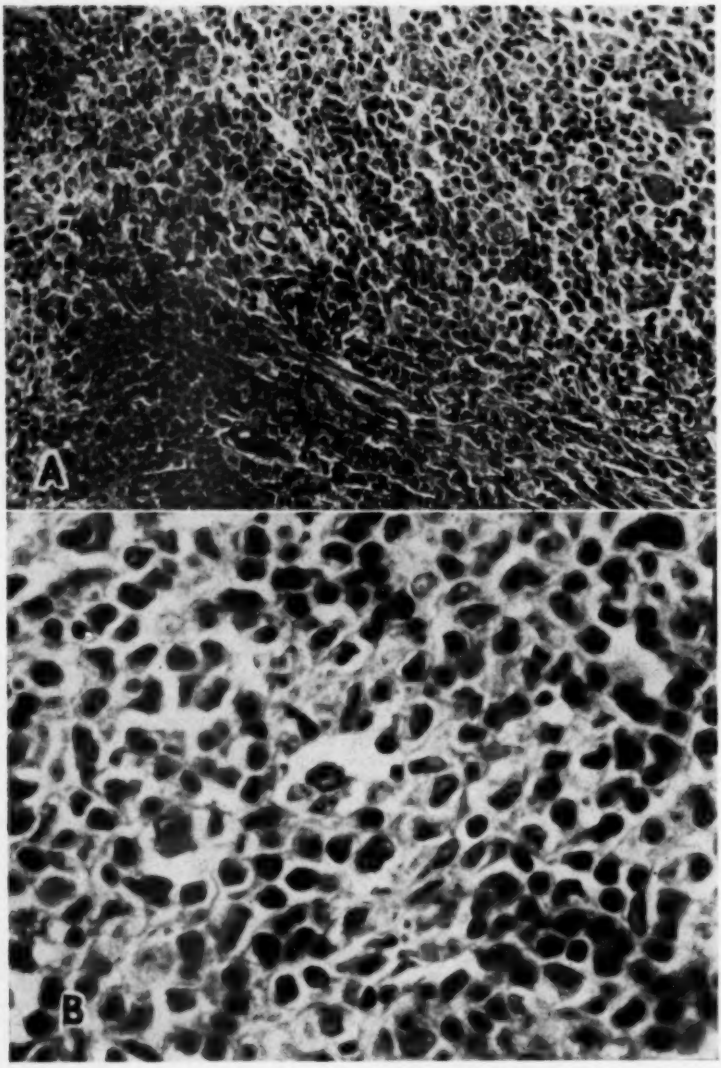

Fig. 3.—*A*, photomicrograph of representative field of a focal nodule in the lung biopsy specimen from one of the cases reported by Farinacci and associates.[30] Note replacement of pulmonary parenchyma by histiocytes interspersed with prominent collections of (eosinophilic) leucocytes. Except for the absence of hemorrhage and necrosis and a tendency toward concomitant fibroblastic proliferation, the picture resembles that of eosinophilic granuloma of bone; × 200. *B*, higher magnification of pulmonary lesion of "eosinophilic granuloma" illustrated in *A*. The smaller dark cells dispersed among the histiocytic cells are young eosinophiles, many of which are uninuclear or bilobed; ×565.

with numerous eosinophiles, and otherwise, by a tendency toward fibrosis in places. Through the courtesy of Dr. Farinacci, I have had the opportunity to study these biopsy sections, and I concur in the impression of pulmonary eosinophilic granuloma and in the view that both cases should be regarded clinically as instances of early chronic disseminated histiocytosis X (S-C), even though the full picture of the disorder had not yet developed (Fig. 3). Timely confirmation of the soundness of this view comes from another comparable key case, the pertinent biopsy material of which I saw recently through the courtesy of Dr. J. H. Childers.[31] This patient, a 24-year-old man, presented pulmonary findings essentially duplicating those described by Farinacci and co-workers, and lung biopsy likewise showed a picture of nodular infiltration by foci of eosinophilic granuloma. A roentgen skeletal survey in this case revealed a rarified defect in a femur, biopsy of which showed a picture indistinguishable from that of eosinophilic granuloma of bone.

As yet, relatively little is known concerning comparable early lesions of disseminated histiocytosis X in internal sites other than the lungs, although their presence may be inferred from the distribution of lesions observed in cases which eventually come to autopsy. With the currently increasing employment, however, of needle biopsy of the bone marrow, liver, and even the spleen as a diagnostic aid, pertinent information of value may be forthcoming presently.

With reference to the pathologic changes observed in fatal cases of acute disseminated histiocytosis X (L-S), a number of additional, detailed protocols of autopsy findings in infants have been recorded [32] within the past several years, but these have not contributed anything fundamentally new. In this connection, however, one significant point not previously emphasized deserves particular mention. This relates to the findings at autopsy in certain instances of chronic disseminated histiocytosis X (S-C) in which terminally the tempo is greatly accelerated, so that the clinical picture before exitus comes to resemble that of the Letterer-Siwe syndrome. In these circumstances it has been observed [33] that the rapidly proliferating histiocytes in the fresh skeletal lesions may become foamy virtually as fast as they are formed (Fig. 4). In fact, such skeletal foci may be distinctly yellow in the gross, obviously reflecting their lipid content, and have a strikingly soft, almost creamy, consistency. It would appear, therefore, that nonlipid histiocytosis is not always a valid or accurate designation for acute disseminated histiocytosis X (L-S) and, collaterally, that pointed reference to the presence or absence of lipid in the pertinent lesions is not really of crucial significance in distinguishing the chronic and acute forms of the disease, respectively.

By the same token, one must perforce revise the previously held view [6a] that the prerequisite for the pathologic diagnosis of Schüller-Christian disease (chronic disseminated histiocytosis X) is the presence of collagenized and lipidized lesions classifiable as "lipogranuloma" or "lipid granuloma" (Fig. 5). The accrued obser-

31. Childers, J. H.: Case report to be published.

32. Levinsky, W. J.: Nonlipid Reticuloendotheliosis: Letterer-Siwe Disease: Report of a Case, Arch. Path. **48**:462, 1949. Schafer.[17]

33. Laymon, C. W., and Sevenants, J. J.: Systemic Reticuloendothelial Granuloma: Comparison of Letterer-Siwe Disease, Schüller-Christian Disease and Eosinophilic Granuloma. Arch. Dermat. & Syph. **57**:873, 1948. Horsfall, F. L., Jr., and Smith, W. R.: Lipoid Granulomatosis, Defects in the Bones, Exophthalmos and Diabetes Insipidus, Quart. J. Med. **6**:37, 1935. Lichtenstein, L.: Personal observation (unreported).

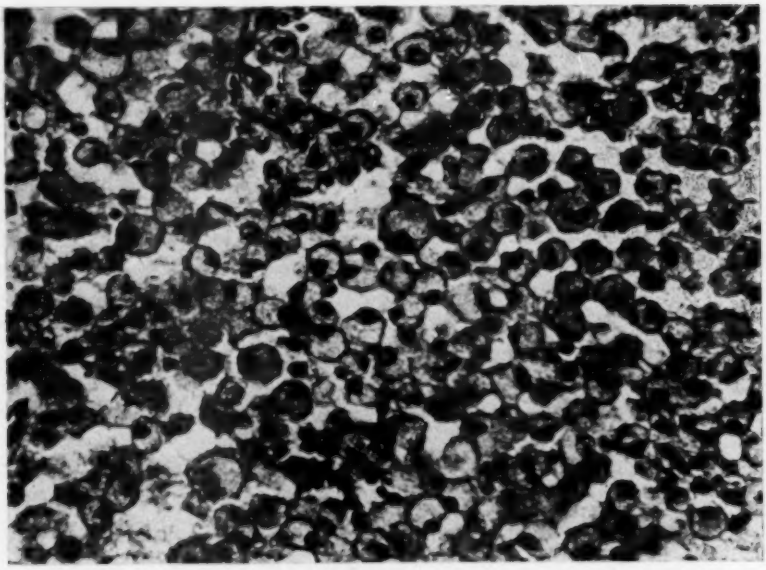

Fig. 4.—Photomicrograph of one of many skeletal lesions appearing terminally in a 3-year-old child presenting the clinical picture of the Letterer-Siwe syndrome. Note the foamy character of the compacted swollen histiocytes; × 325.

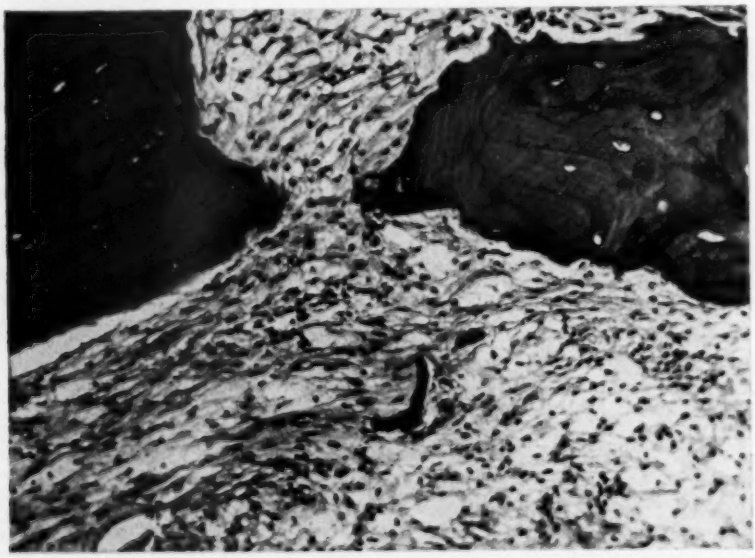

Fig. 5.—Photomicrograph of an old sclerotized lesion of a tibia in an adult who presented no clinical evidence of extraskeletal involvement. Note the presence of numerous lipophages and collagen-forming fibroblasts in the tissue between the sclerotic trabeculae of spongy bone. This is the picture which is often referred to as lipoid granulomatosis; × 200.

vations already cited have made it increasingly evident that the initial pathologic picture is essentially that of an inflammatory histiocytosis (or granulomatosis), which may or may not be accompanied by intense eosinophilic reaction, but which, in any event, exhibits no significant tendency at the outset toward lipidization. The picture of lipogranuloma so-called, viewed in proper perspective, apparently represents the late, or end, phase of the evolution of this histiocytic lesion, and it has been given undue prominence and importance in the past through the observation largely of necropsy material.[18] The fibrosis observed at this stage may be plausibly interpreted as an expression of healing, while the conversion of some of the histiocytes to lipophages seems clearly to represent a secondary change. Nor does it appear at all necessary to invoke a theory of cellular derangement of lipid metabolism or a metabolic disorder of the reticulum cell ("essential xanthomatosis"), as Thannhauser has postulated,[13] to explain this secondary lipidization. The latter may also be observed in many other diverse skeletal lesions, e. g., nonosteogenic fibroma, pigmented villonodular synovitis, fibrous dysplasia of bone, treated giant cell tumor, and chronic osteomyelitis, among others. Actually, as previously noted,[6a] the genuine cholesterol analogue of Niemann-Pick disease and Gaucher's disease is not the condition under discussion at all, but is rather xanthoma tuberosum multiplex.

Another idea in regard to Schüller-Christian disease so-called, which also requires a fresh approach, is that this condition represents a rather ill-defined, miscellaneous clinical category.[6a] It is true that the clinical picture may vary considerably from case to case. However, virtually all the apparently miscellaneous, as well as unusual, cases fell logically into line within a unified framework of reference (Table 2), if one adopts a broader interpretation of Schüller-Christian disease so-called as the clinical expression of chronic disseminated histiocytosis X rather than "lipoid granulomatosis," and if one recognizes, as noted, that the tempo and severity of the disease may range within wide limits and that a number of sites may be predominantly involved in varying combinations. Thus, at one extreme there are patients, usually younger children, whose ultimate prognosis is serious, although the total duration of illness may be measured in years rather than months, and at the other extreme there are patients, usually adults, whose complaints are so mild as to escape recognition for a long time. Furthermore, as also noted, cases of acute or subacute disseminated histiocytosis X (L-S) may become chronic (S-C), and, conversely, cases of chronic disseminated histiocytosis X (S-C) may exhibit an acute exacerbation (L-S). When destructive skeletal foci appear, either initially or later in the course of the disease, these commonly have the character of eosinophilic granuloma, provided that they are examined within several months of the time of their appearance. Similarly, when fresh lesions appear in such extraskeletal sites as the skin, the oral mucosa (the gingiva and palate particularly), the anogenital region, the lymph nodes, and the lungs, these, too, can present the pathologic picture of what may also be called eosinophilic granuloma. Apart from these factors, the clinical coloring of any individual case, as noted, is determined by the particular localization of the significant lesions, the major types of involvement being skeletal, pulmonary, cutaneous and hypophyseal (or cerebral).[34]

34. Hewer, T. F., and Heller, H.: Non-Lipid Reticulo-Endotheliosis with Diabetes Insipidus: Report of a Case, J. Path. & Bact. **61**:499, 1949. Cureton, P. J. R.: A Case of Intracerebral Xanthomatosis with Pituitary Involvement, ibid. **61**:533, 1949.

As for the Christian triad, which has received so much attention in the past, it seems hardly necessary at this late date to reiterate that skeletal defects need not be situated in the calvarium and that many patients likewise fail to exhibit either exophthalmos or diabetes insipidus. For that matter, even when the full Christian triad is present, it does not necessarily establish a diagnosis of chronic disseminated histiocytosis X (S-C), inasmuch as it has also been observed in the acute form of the disease (L-S). As for diabetes insipidus in particular, we now recognize that its manifestations may result directly from involvement of the pituitary, its infundibulum or the tuber cinereum, in the absence of skull defects. In fact, skeletal defects in general should no longer be considered a *sine qua non* for diagnosis of the disease, since they may be absent, or, if they do appear, they may not become manifest until lesions in extraskeletal sites are already well established.

SUMMARY

This article represents in essence a critical review of the subject indicated in the title, covering especially the period from 1944 to date, and an attempt at comprehensive reorientation on the basis of significant recent observations. These have lent additional strong support to the concept that the conditions previously designated eosinophilic granuloma of bone, "Letterer-Siwe disease" and "Schüller-Christian disease" are interrelated manifestations of a single malady. The name "histiocytosis X" is suggested as a provisional broad general designation for this nosologic entity. The recognized types of clinical involvement can be grouped under this general heading and still be differentiated from one another so that useful distinctions having a bearing on treatment and prognosis are maintained.

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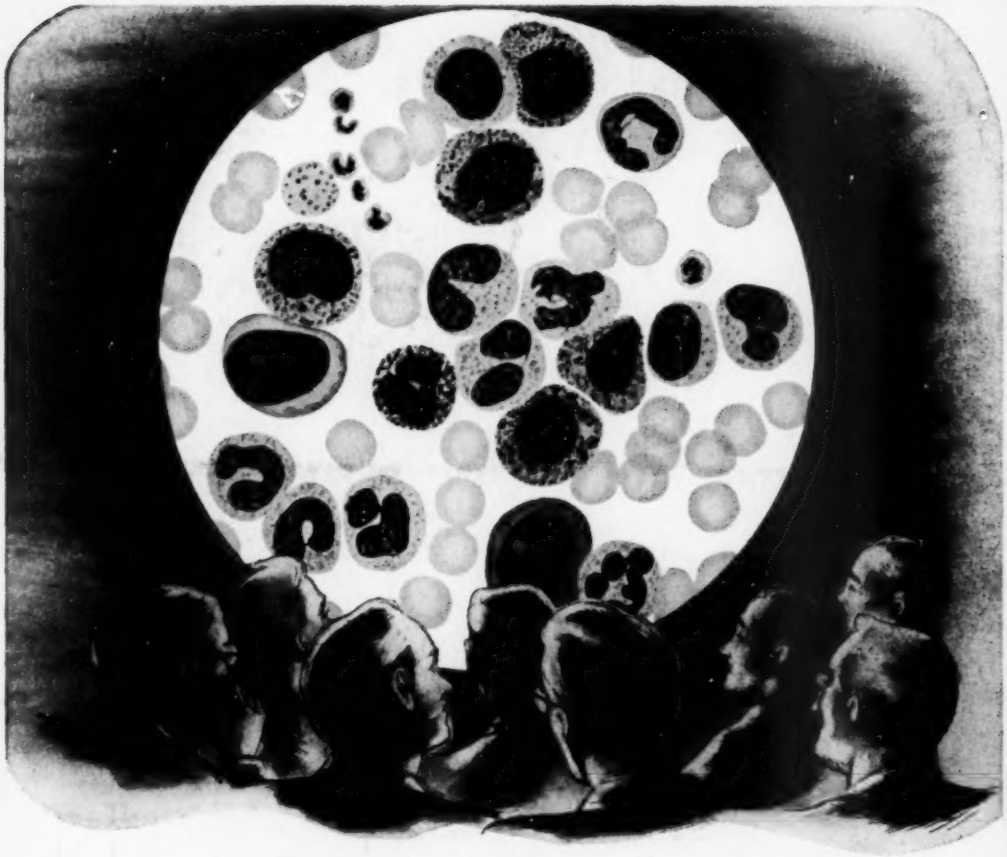

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